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Alternative Splicing and Cycling Kinetics of Myosin Change During Hypertrophy of Human Smooth Muscle Cells

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Abstract We investigated in vivo expression of myosin heavy chain (MHC) isoforms, 17 kDa myosin light chain (MLC_{17}), and phosphorylation of the 20 kDa MLC (MLC_{20}) as well as mechanical performance of chemically skinned fibers of normal and hypertrophied smooth muscle (SM) of human myometrium. According to their immunological reactivity, we identified three MHC isoenzymes in the human myometrium: two SM-MHC (SM1 with 204 kDa and SM2 with 200 kDa), and one non-muscle specific MHC (NM with 196 kDa). No cross-reactivity was detected with an antibody raised against a peptide corresponding to a seven amino acid insert at the 25K/50K junction of the myosin head (a-25K/50K) in both normal and hypertrophied myometrium. In contrast, SM-MHC of human myomatous tissue strongly reacted with a-25K/50K. Expression of SM1/SM2/NM (%) in normal myometrium was 31.7/34.7/33.6 and 35.1/40.9/24 in hypertrophied myometrium. The increased SM2 and decreased NM expression in the hypertrophied state was statistically significant ($P < 0.05$). MHC isoform distribution in myomatous tissue was similar to normal myometrium (35.3/35.3/29.4). In vivo expression of MLC_{17a} increased from 25.5% in normal to 44.2% in hypertrophied ($P < 0.001$) myometrium. Phosphorylation levels of MLC_{20} upon maximal Ca^{2+}-calmodulin activation of skinned myometrial fibers were the same in normal and hypertrophied myometrial fibers. Maximal force of isometric contraction of skinned fibers (pCa 4.5, slack-length) was 2.85 mN/mm^2 and 5.6 mN/mm^2 in the normal and hypertrophied state, respectively ($P < 0.001$). Apparent maximal shortening velocity ($Vmax_{app}$, extrapolated from the force-velocity relation) of myometrium rose from 0.13 muscle length s^{-1} (ML/s) in normal to 0.24 ML/s in hypertrophied fibers ($P < 0.001$). J. Cell. Biochem. 64:171–181.

Key words: alternative splicing; myometrium; myoma; smooth muscle; myosin heavy chains; myosin light chains; hypertrophy

The myosin molecule of all muscle types is composed of two heavy chains (MHC) which are associated with two types of light chains each [1]. At least seven different genes coding for MHC and seven genes coding for MLC exist in striated muscle [2, 3]. They switch myosin subunit expression mainly by changing transcriptional activity of different genes namely in a developmental and tissue-specific manner or in response to different environmental demands [3]. It could be demonstrated that the myosin isoforms expressed determined both the energetical and mechanical characteristics of different types of cross-striated muscles [3]. MHC and MLC isoforms of smooth muscle (SM) are produced by both expression of different genes and differential splicing of single genes [4]. In smooth muscle cells (SMC) three different genes coding for MHC are expressed, namely one smooth-muscle specific (SM-MHC) and two genes coding for non-muscle (NM) MHC. The single SM-MHC gene transcript is alternatively spliced at both the 3′- and at the 5′-end of the gene. Alternative splicing of a 39 nucleotide (nt) exon of SM2 (200 kDa) at the most 3′-terminus generates SM1 (204 kDa) [5–7]. 5′-splicing is accomplished by excision of 21 nt (7 amino acids) near the ATP-binding 25K/50K-

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junction [8–10]. As demonstrated by mRNA analysis about 85% of rat bladder MHC contained the 25K/50K-insertion while MHC of rat aorta and myometrium contained very low levels of SM-MHC with 25K/50K-insertion [8]. Two genes coding for NM-MHC are expressed in SMC designated as NM-MHCA (196 kDa) and NM-MHCB (198–200 kDa) [11, 12]. NM-MHCB is expressed in the embryonic SMC (designated, therefore, as SMemb either) [13, 14], in proliferating SMC during the formation of vascular lesions [15] and in culture [11]. In the adult state, myometrial cells express NM-MHCA in addition to SM-specific MHC isoforms [16].

SMC express two essential MLC isoforms with 17 kDa by alternative splicing, namely MLC_{17a} and MLC_{17b}. In smooth muscle cells of human [17] and chicken [18] MLC_{17b} differs from MLC_{17a} by inclusion of an insert of 44 (human) or 39 (chicken) nt located in the C-terminus. MLC_{17a} is also expressed in non-muscle cells whereas MLC_{17b}, which contains the insertion, seems to be SM specific [19]. Two isoforms of the 20 kDa phosphorylatable MLC, designated as the MLC_{20A} and MLC_{20B} with increasing acidity [20, 21] have been identified in SMC. Phosphorylation of the MLC_{20} by Ca^{2+}-calmodulin dependent myosin light chain kinase is a key event in the initiation of smooth muscle contraction and the extent of MLC_{20} phosphorylation determines tension development [for review see 22].

Little is known about regulation of alternative splicing as well as the functional consequences of alternatively spliced MHC and MLC isoforms in the SMC. We could recently demonstrate that steroid hormones are involved in the regulation of myosin subunit expression: testosterone favoured the expression of SM1 and 5′-inserted MHC while estrogen normalized ovarectomy-induced diminished MLC_{17a} expression [26]. MHC containing the 25K/50K-insertion revealed a higher ATPase activity and moved actin filaments faster in in vitro motility assays than MHC without 5′-insert [10]. In contrast, smooth muscle fibers from urinary bladder containing 5′-inserted MHC [8] revealed a shortening velocity similar to myometrium fibers [23] having no 5′-inserted MHC [8]. MLC_{17} isoforms have been correlated with SM energetics and contractility. There is a positive correlation between MLC_{17a} expression and both shortening velocity [23] and ATPase activity [24] but a correlation between MLC_{17} isoforms and the velocity of actin filament sliding in the in vitro motility assays is controversial [compare 10 and 19].

In order to elucidate the physiological function of myosin subunit isoforms in the smooth muscle we investigated MHC and MLC expression and contractile behaviour of normal and hypertrophied SMC. This approach seems to be justified since it is known that MHC and MLC changed during SM hypertrophy [16, 25]. Analysis of mechanical alterations and myosin subunit expression provide insight into the physiological function of different myosin isoforms in the SMC. In this study we investigated both, alternative splicing and gene transcription of the different MHC and MLC isoforms as well as mechanical (length-tension ratio, isometric tension, force-velocity ratio) characteristics of normal and hypertrophied human myometrium from pregnant patients. It is well established that myometrial SMC undergo hypertrophy during pregnancy [27]. We found that the accelerated cross-bridge cycling kinetics and enhanced contractility of the hypertrophied SMC of human myometrium correlated with upregulation of MLC_{17a} and down-regulation of NM-MHC.

MATERIALS AND METHODS

Treatment of Tissue

Normal (non-hypertrophied) myometrium and myomatous tissue were obtained from non-pregnant patients during hysterectomy. Hypertrophied myometrium was excised from pregnant patients during caesarean section. All tissue specimen derived from the Isthmus uteri. Pregnant patients were 30.8 ± 3.4 years old, mean age of non-pregnant patients was 40.9 ± 5.1 years. Experiments were performed with the understanding and consent of each individual. The study had been approved by the local Ethical Committee. Tissue specimen were either immediately frozen in liquid nitrogen and stored at −80°C, or chemically skinned according to the following method: excised tissue was divided in small fiber bundles (about 1 mm thick, 6 mm length) and incubated for 30 min in (mM): EGTA 5, imidazole 20, KCl 50, sucrose 110, dithioerythrole 2, pH 7.4, on ice. Tissue samples were subsequently incubated in the same solution containing in addition 1% Triton X-100 for 4 h on ice. Skinned myometrial fibers were then transferred into a solution

containing (mM): imidazole 20, EGTA 4, ATP 7.5, M_gC_12 10, NaN_3, pH 6.7, and 50% glycerol for 1 h on ice and subsequently stored at −20°C. All skinning solutions contained 10 µM leupeptine as protease inhibitor.

Analysis of In Vivo Myosin Heavy Chain (MHC) Isoform Expression

To analyze the in vivo MHC expression, frozen uteri were powdered in liquid nitrogen, mixed with 15% TCA, and denatured for 20 min on ice. The homogenate was centrifuged and TCA was extracted from the protein pellet with ether/ethanol (80/20 v/v). The muscle powder was stored at −20°C under N_2. For analysis the muscle powder was boiled for 1 min in SDS-sample buffer (5% SDS, 50 mM Tris/HCl, pH 6.8) and cleared by centrifugation. Protein of the supernatant (50 µg) was analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) [28]. In short, we used a 4% stacking gel, 5% separation gel both containing 25% glycerol. Gels were run for 6 h (40 mÅ per gel) at 15°C under continuous circulation between upper and lower electrode buffer. Gels were stained for protein with Coomassie blue R250, destained overnight, and the MHC were evaluated densitometrically (ScanPack, Biometra, Germany).

Antibody Production and Affinity Purification

A peptide antibody specific for the 25K/50K-insertion of rat smooth muscle MHC (5′-insert) was generated based on the amino acid sequence "QGPSFAY" [compare 8]. At the amino terminus of the peptide N-chloroacetylglycine was added to facilitate cross-linking to carriers. The peptide was synthesized by the solid-phase method, purified, coupled to keyhole limpet hemocyanine, and used for immunization of rabbits as described previously [29]. The resulting antibody fractions were purified on a peptide-affinity column. For this a packed gel of w-aminohexylagarose (0.5 ml) was activated with a 10-fold molar excess of 2-iminothiolane (Traut reagent) for 20 min at room temperature. Activated gel was poured into a column and was extensively washed with 0.1 M $NaHCO_3$, pH 8.0. A solution of 1 ml of N-chloroacetylglycine-QGPSFAY (8 mg) was mixed with the activated gel and immobilized by incubation on a rotating wheel for 3 h at room temperature. The peptide-resin was then incubated for 1 h with 5 ml of 40 mM iodoacetamide to derivatize any remaining sulfhydryl groups. Following extensive washing, the peptide-resin was gently mixed with 5 ml of antiserum diluted 2-fold in 10 mM Tris/HCl-buffer (pH 7.4) containing 200 mg of bovine serum albumin and 0.1 mM phenylmethylsulfonyl fluoride by rotation at 4°C overnight. To elute bound antibodies, 50 mM glycine/HCl-buffer (pH 2.5) was used and the pH was immediately adjusted to pH 7.4. The fractions exhibiting absorption at 280 nm were pooled and stored at −80°C. The a-25K/50K strongly cross-reacted with MHC derived from rat urinary bladder which contained the 5′-insertion but not or only weakly with rat myometrium having MHC mainly without 5′-insertion [8] (not shown).

Western-Blot Analysis

Myosin heavy chain (MHC) isoforms were analysed by a semi-dry Western-blot technique. To identify MHC isoforms we used a smooth muscle MHC-specific polyclonal antibody directed against chicken gizzard MHC (a-SM) raised in rabbit, the above-mentioned peptide antibody raised against the 25K/50K-insert (a-25K/50K), and a polyclonal antibody raised against the rod portion of non-muscle myosin (a-NM). a-SM and a-NM were kindly provided by Prof. Gröschel-Stewart, Darmstadt, Ger-

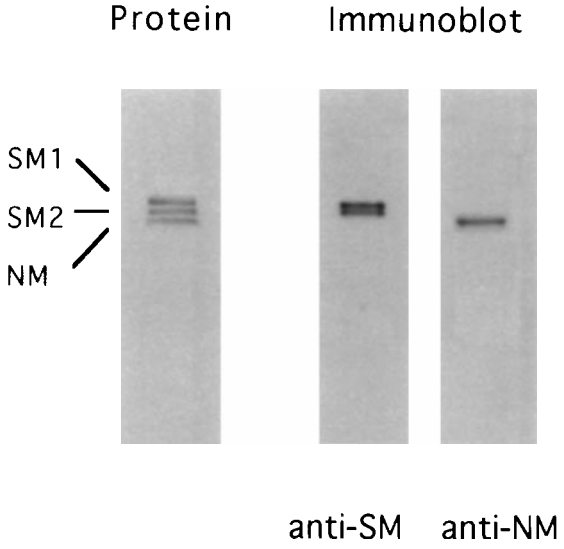

Fig. 1. Analysis of in vivo myosin heavy chain (MHC) expression in normal human myometrium by SDS-PAGE according to Carraro and Catani, 1983 [28]. Gels were stained for protein with Coomassie blue R-250 (Protein, left) or analysed by immunoblot using antibodies specific for smooth muscle MHC (anti-SM) and non-muscle MHC (anti-NM). SM1 and SM2 refer to the 204 and 200 kDa smooth muscle MHC, respectively. NM refer to the 196 kDa non-muscle MHC.

many [30]. Proteins were transfered from SDS-gels to hybond-ECL (Amersham, Arlington Heights, IL) (90 min, 0.8 mÅ/cm^2), incubated with the primary antibody (2 h, room temperature) and subsequently with the secondary peroxidase-conjugated antibody (antirabbit) for 1 h at room temperature. Proteins were visualized by enhanced chemoluminescence reaction kit (ECL, Amersham) using X-Omat (Kodak) as X-ray film.

Analysis of Myosin Light Chains (MLC)

Phosphorylation of the 20 kDa MLC and the distribution of the 17 kDa MLC isoforms (MLC_{17a} and MLC_{17b}, with decreasing order of acidity) of skinned uteri and TCA extracts (in vivo expression) was analyzed by 2-dimensional gel electrophoresis (2D-PAGE). Isoelectric focusing (first dimension) was performed in glass capillaries (12.5 cm length, 1 mm inner diameter) using the pH gradient 4.5–5.4 (Pharmalytes, Pharmacia, Sweden). The gels were run overnight at 600 V constant for the first dimensional separation. The second dimension was a SDS-disc electrophoresis [31]. Slab gels were 10.5 × 9.5 cm, 1 mm thick. To measure Ca^{2+} activated phosphorylation skinned fibers were incubated under isometric conditions for 30 min at room temperature either in pCa 4.5/1 µM calmodulin or in pCa 8/1 µM calmodulin (for composition of solutions see below). The reaction was stopped by immersion of the fibers in ice-cold 15% TCA and the MLC were subjected to 2D-PAGE as described above. The gels were silver stained (SilverStain, Bio-Rad, Richmond, CA) and the MLC were scanned densitometrically.

Mechanical Analysis

All mechanical experiments were performed with normal and hypertrophied myometrial skinned fibers (about 200 µm diameter, 4–6 mm length). Fibers were mounted horizontally between a force transducer (AME-AE 801, Senso-Nor, Horten, Norway) and a length step generator with a celluloid acetone glue. Relaxation solution contained (mM): HEPES (50), ATP (10), creatine phosphate (10), $MgCl_2$ (12.5), NaN_3 (5), EGTA (5), DTE (1), KCl (12.5), calmodulin (0.001), pH 7. Contraction solution was the

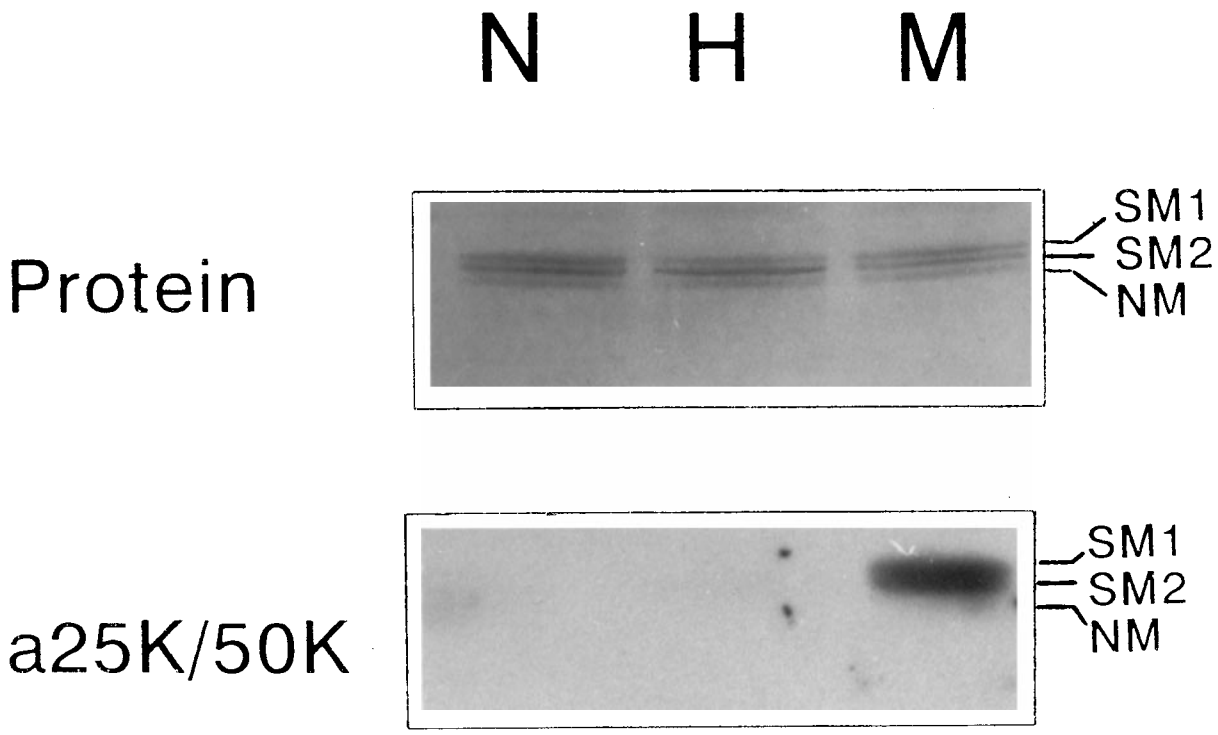

Fig. 2. Western-blot analysis of MHC of normal and hypertrophied SMC of human myometrium and of myomatous (M) tissue using a peptide antibody (a-25K50K) raised against the rat 5′-insertion QGPSFAY. Top: Protein stain (Coomassie-blue). Bottom: Western-blot. SM1 and SM2 refer to the 204 and 200 kDa smooth muscle MHC, respectively. NM refer to the 196 kDa MHC. SM-MHC of myomatous but not of and hypertrophied strongly reacted with a25K/50K.

same as relaxation solution except that EGTA was substituted by 5 mM CaEGTA. Free Ca^{2+} concentrations were calculated using the computer program of Fabiato and Fabiato, 1979 [32]. Ionic strength was adjusted to 130 mM with KCl.

The fibers were mounted in relaxation solution and length was adjusted such that resting tension was just threshold. This was about 0.75 L_0 of both normal and hypertrophied myometrial fibers (L_0 is the length were maximal force is generated). Subsequently the fibers were transferred into maximal Ca^{2+} activation solution (pCa 4.5). Force-velocity relations were determined by isotonic quick releases under "constant load" at 22°C. Load clamping for isotonic shortening was achieved by rapidly changing the mode of operation of the puller from fiber-length control during isometric steady state tension. The force during isotonic contraction was held constant by the controlled motion of the puller which followed the contracting fiber with the appropriate velocity. The force control mode was maintained for 250 ms. The velocity of the length step was determined with an optoelectronical position detector (Hamamatsu, Japan). Both force and velocity signals were displayed on a storage digital oscilloscope and analyzed with an IBM compatible PC. The relation between shortening velocity and force during isotonic contraction was analyzed using a linearized form of the Hill, 1938 [33] equation:

$$(P + a)(v + b) = (P_0 + a)b$$

where v is the shortening velocity, P is the force during isotonic contraction, Po is the maximal isometric steady-state force and a and b are constants.

Statistics

Statistical analysis were performed using the commercially available statistic program "epistat" on an IBM compatible PC (means, standard error [SEM], student's T-test for unpaired

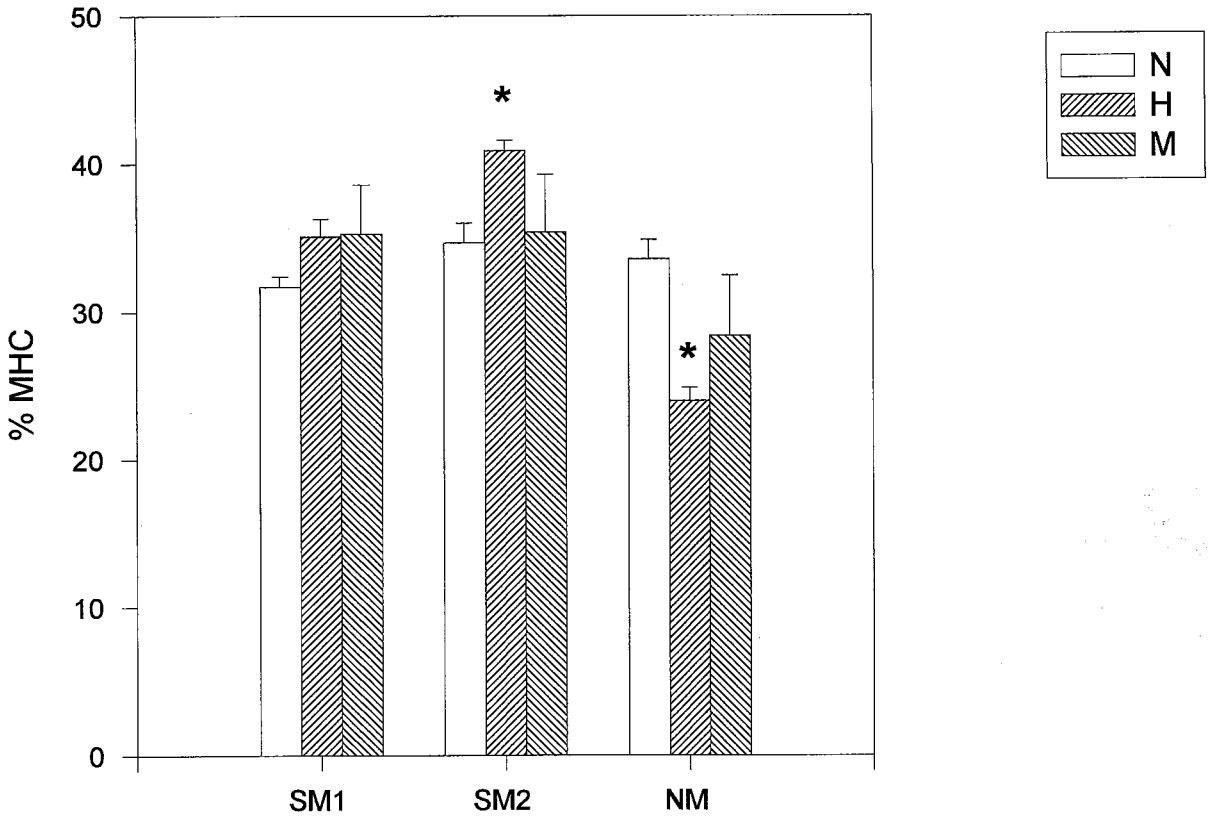

Fig. 3. Statistical analysis of MHC expression evaluated by densitometrical scanning of the Coomassie-stained protein bands of normal (N), hypertrophied (H), and myomatous (M) human myometrium. SM1 and SM2 refer to the 204 and 200 kDa smooth muscle MHC, respectively. NM refer to the 196 kDa MHC. Values are means ± SEM. *$P <$ 0.05. Six patients per group.

values). All values are expressed as means ± SEM with number of patients/number of fibers per patient investigated in parenthesis.

RESULTS

In Vivo Expression of MHC Isoforms

MHC of normal and hypertrophied human myometrium as well as human myomatous tissue were analyzed according to their apparent molecular mass (Mr) (relative mobility in the SDS-gel) and immunological reactivity (Western-blot analysis) using specific antibodies raised against MHC of smooth muscle (a-SM, a-25K/50K) and non-muscle (a-NM). Three myosin heavy chains (MHC) could be observed on the protein level in human myometrium and myomatous tissue having Mr of 204 kDa MHC (SM1), 200 kDa (SM2), and 196 kDa (NM) (Figs. 1, 2). As demonstrated in a representative analysis of normal tissue in Figure 1, SM1 and SM2 cross-reacted selectively with a-SM while NM cross-reacted selectively with a-NM (Fig. 1). According to its Mr and immunological reactivity, NM was identified as the NM-MHCA.

MHC of both normal and hypertrophied myometrial tissue showed no detectable immunoreactivity with a25K/50K (Fig. 2). In contrast, SM-MHC, but not NM-MHC, of myomatous tissue strongly reacted with a-25K/50K (Fig. 2). The peptide antibody which has been raised against the rat 5′-insert obviously recognized also the 5′-insertion of human SM-MHC. This suggests a close homology between the rat and human 5′-insert.

As analyzed densitometrically, normal myometrium expressed mean values of 31.7/34.7/33.6% (SM1/SM2/NM, respectively; 6 patients). Hypertrophied myometrium (6 patients) revealed 35.1/40.9/24%, i.e., significantly increased relative amounts of SM2 and decreased NM ($P < 0.05$) (Fig. 3). Expression of MHC in myomatous tissue (6 patients) was similar to that of normal myometrium (35.3/35.3/29.4%) (Fig. 3).

Expression of MLC_{17}

Two myosin light chain isoforms with 17 kDa could be observed upon analysis of intact and skinned myometrium by 2D-PAGE, namely the more acidic MLC_{17a} and the more alkaline MLC_{17b} (Fig. 4). Densitometrical scanning of both MLC_{17} isoforms revealed a relative amount of MLC_{17a} of 25.5% [6] in normal SMC. This increased significantly ($P < 0.001$) to 44.2% [6]

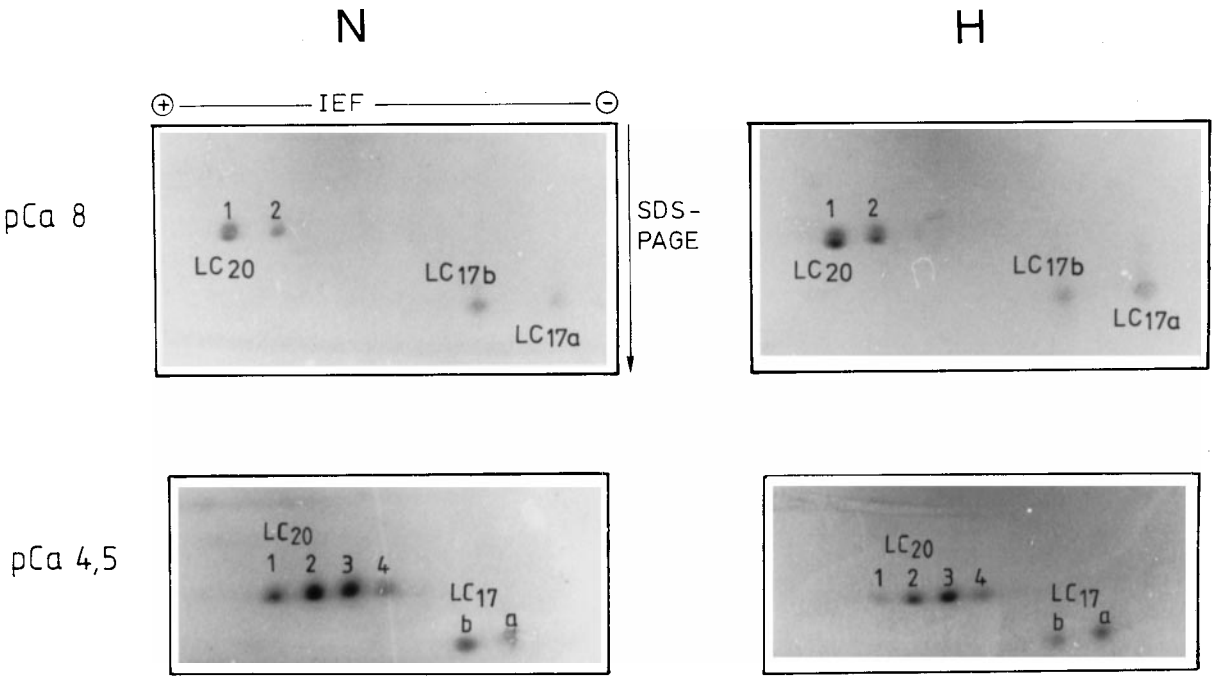

Fig. 4. Analysis of 20 kDa myosin light chain forms (LC_{20} 1, 2, 3, 4) and the LC_{17} isoforms ($LC_{17a,b}$) in skinned fibers from normal and hypertrophied human myometrium by 2D-PAGE. Gels were stained with silver. Fibers were incubated for 30 min in relaxation solution (pCa 8; top) or in maximally Ca^{2+}-calmodulin-containing contraction solution (pCa 4.5; bottom).

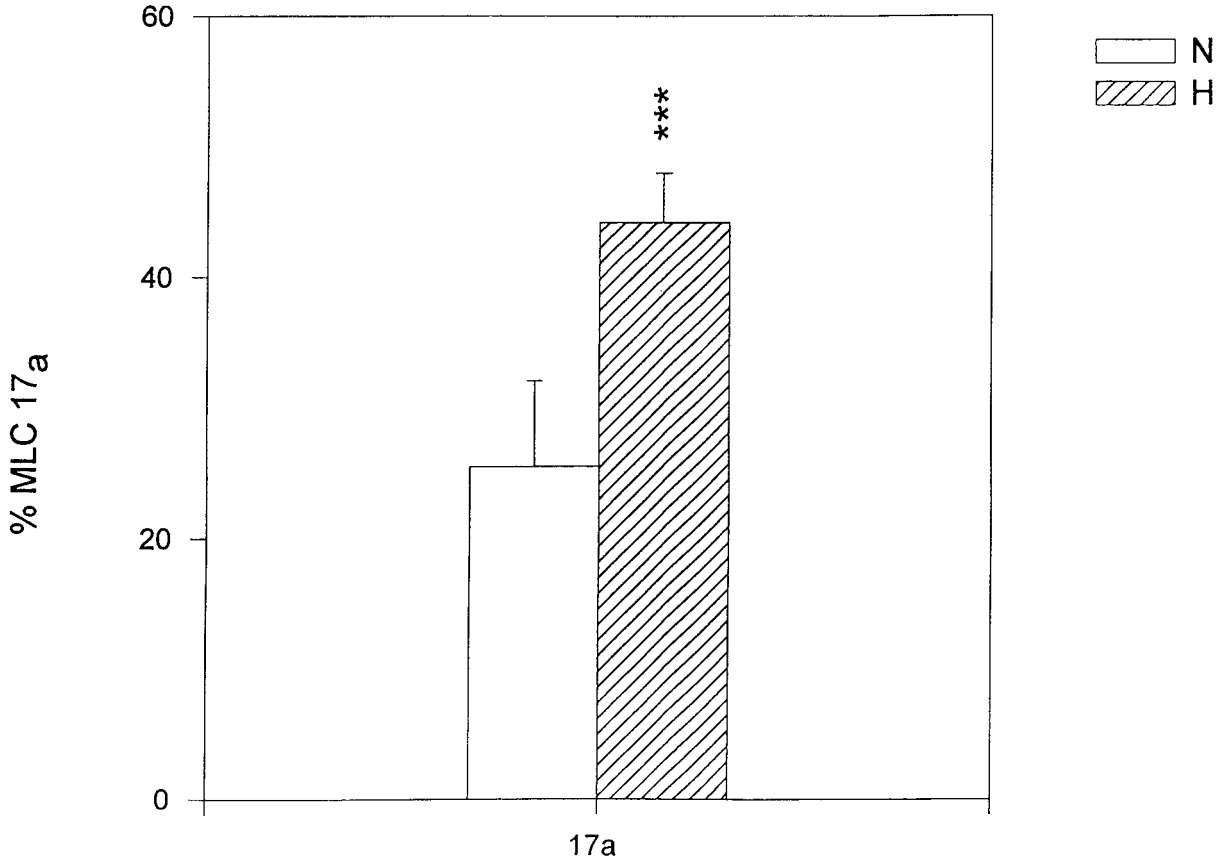

Fig. 5. Densitometrical analysis of MLC_{17a} (expressed in % of whole MLC_{17}) of normal (N) and hypertrophied (H) human myometrium. Values are means ± SEM. ***$P < 0.001$.

in hypertrophied myometrial cells (Figs. 4, 5). MLC isoform distribution in chemical skinned and intact myometrium was the same (not shown).

Analysis of MLC_{20} Phosphorylation

Four MLC20 protein forms could be resolved in myometrial fibres designated with increasing acidity with numbers 1–4 (Fig. 4). We omitted the evaluation of the phosphorylation level in "mol Pi/mol MLC_{20}." Instead, we expressed MLC_{20} protein spots 1–4 in percent (%) of whole MLC_{20} spots and compared the different MLC_{20} forms of normal and hypertrophied fibers under relaxing (Fig. 6a) and activating (Fig. 6b) conditions. After 30 min incubation in relaxation solution, mainly the alkaline (unphosphorylated) spots 1 and 2 could be observed. The relative amounts of MLC_{20} forms were similar in normal and hypertrophied myometrial fibers (6 fibers were investigated per group) (Fig. 6a). Upon Ca^{2+} activation, the alkaline MLC_{20} forms decreased in favour of the more acidic MLC_{20} forms 3 and 4 (phosphorylated forms) (Fig. 6b). The relative amounts of MLC_{20} forms were similar in both normal and hypertrophied upon Ca^{2+}-activation.

Mechanical Studies

Relative length-tension ratios of normal and hypertrophied Triton-skinned fibers were the same. Slack length (fiber length where passive tension was just threshold) was about 0.75 L_0 (L_0 is the fiber length where maximal active tension was achieved). Maximal force (pCa 4.5) per cross section obtained at slack length was significantly ($P < 0.05$) higher in hypertrophied than in normal fibers, namely 2.85 ± 0.3 mN/mm^2 (5/2) and 5.6 ± 0.4 mN/mm^2 (5/2) in normal and hypertrophied fibers, respectively.

Force-velocity relations of skinned human myometrial fibers were obtained by applying isotonic quick releases under constant load conditions at slack length during steady state maximal isometric tension (pCa 4.5). Shortening velocities were measured between 25–50 ms

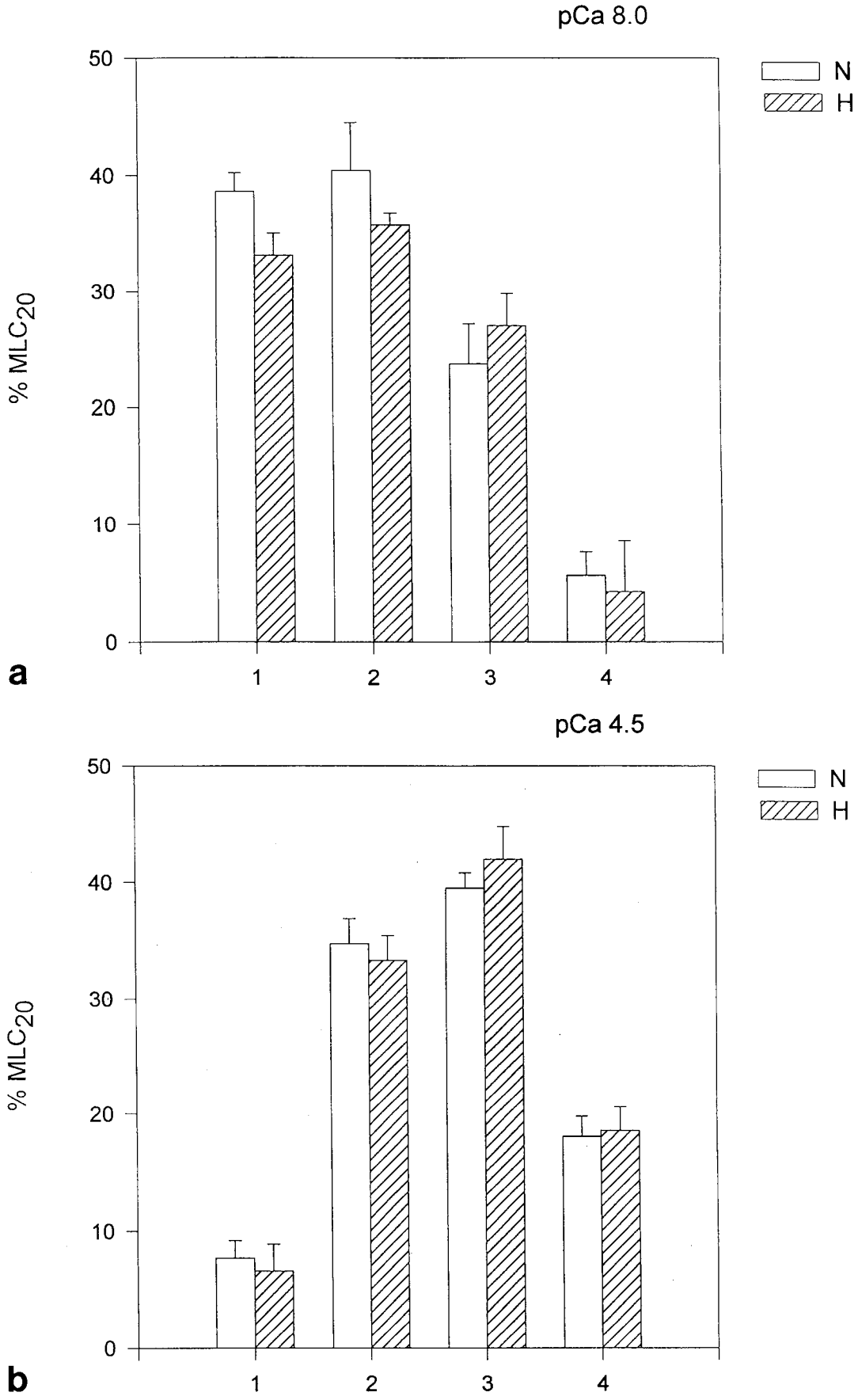

Fig. 6. Statistical analysis of MLC_{20} forms of normal (N) and hypertrophied (H) human myometrium under relaxation condition (pCa 8; **a**) and maximally calcium activation (pCa 4.5; **b**). Values are means ± SEM.

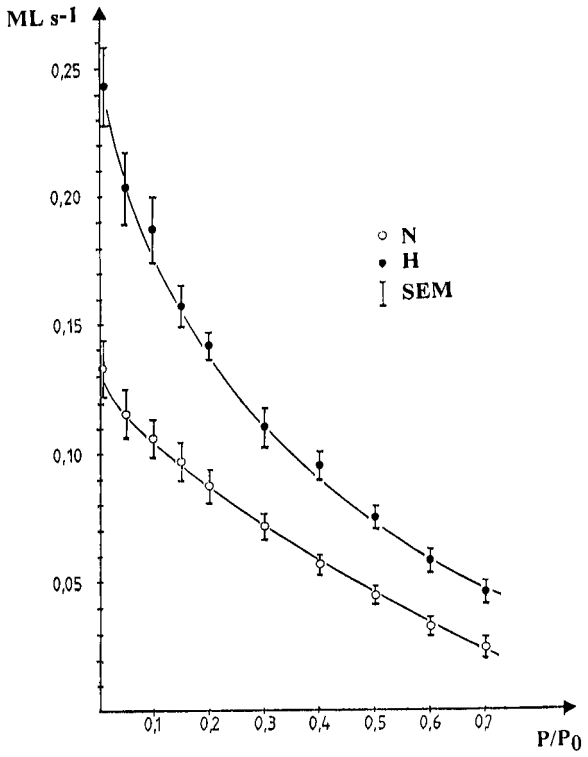

Fig. 7. Force-velocity relations based on load-clamp recordings of Triton-skinned fibers prepared from normal (N; open circles) and hypertrophied (H; filled circles) human myometrium. Values are means ± SEM (6 patients per group, 5 fibres each). Force is expressed as fractional force were P is force during isotonic release and P0 is maximal isometric force. Velocity is expressed as muscle length s^{-1} (ML/s).

after the quick release. As extrapolated from the force-velocity relations (Fig. 7) to zero load, apparent maximal shortening velocity ($V_{max-app}$) of normal fibers was 0.13 + 0.01 muscle length (ML) s^{-1} (6/5). Skinned fibers obtained from hypertrophied myometrium increased to 0.24 ± 0.015 MLs^{-1} (6/5) being significantly different ($P < 0.001$; 6 patients/5 fibers per patient).

DISCUSSION

Myosin subunit expression changed in the hypertrophied SMC of the human myometrium: NM was down-regulated while the 3′-inserted SM-MHC (SM2) increased. We found no detectable expression of SM-MHC with the 5′-insertion in the hypertrophied myometrial cells. However, a-25K/50K strongly reacted with SM-MHC from human myomatous tissue. This observation demonstrates that the peptide antibody raised against the rat 5 insert has the capacity to recognize the human 5′-insertion. Both species may, therefore, share a high degree of sequence homology of the 5′-insertion.

Whether alternatively spliced SM-MHC regulate contraction is still a matter of debate. It has been suggested that alternative splicing in the 25K/50K-domain regulates shortening velocity of SM since 5′-insertion of SM-MHC increased actin-activated ATPase activity and the velocity of actin filament sliding in the in vitro motility assay [10]. However, while about 85% of SM-MHC mRNA of the rat bladder contained the 5′-insertion [8] it has the same shortening velocity as the myometrium [42] having only minor amounts of 5′-inserted SM-MHC [8]. Furthermore, although rat myometrium and aorta contain comparable low levels of the 5′-insertion [8] they revealed very different shortening velocities, the rat aorta being about one third of the myometrium [23]. We found an increased contractility but no cross-reactivity of MHC from normal and hypertrophied myometrium with a-25K/50K. These results demonstrate that contractility of SMC can be modulated without 5′-splicing of MHC and that the presence of 5′-inserts is not necessarily associated with high shortening velocity.

Likewise, the role of 3′-spliced SM-MHC isoforms in SM contraction is still far from being clear. The hypertrophied myometrial cell increased its contractility irrespective of the direction of MHC distribution (change of MHC in favour of SM1 [16] or SM2 [this study]). Furthermore, during the first post partum period expression of SM2 is induced in the myometrial cell of the rat but there was no change in contractility. In the later post partum period shortening velocity declines while SM-MHC expression persisted [16].

Besides SM-MHC splicing variants, at least two more MHC genes are expressed in SMC [11, 12]. Since these MHC genes are expressed in non-muscle cells either they have been designated as non-muscle MHC (NM-MHC). NM-MHC have been designated as NM-MHCA with 196 kDa and NM-MHCB with 198–200 kDa [11, 12]. NM-MHCB is also designated as SMemb since it is expressed in embryonic SMC [13, 14]. Reexpression of SMemb in the hypertrophied human myometrium could be ruled out since the 200 kDa protein band cross-reacted with a-SM but not with a-NM. In addition, it revealed no cross-reactivity with an anti-body raised against SMemb (Cavaille, personal communication).

We cannot rule out the possibility that down-regulation of NM-MHC is involved in the increased contractility of the hypertrophied SMC: In both, the animal [16] and human model, NM-MHC declined during myometrial hypertrophy in favour of SM-specific MHC. The contribution of an increased relative proportion of SM-MHC to the enhanced contractility, however, seems to be small. This assumption came from the observation that $Vmax_{app}$ of the hypertrophied SMC of human myometrium increased by about 85% while the relative amount of SM2 and the whole amount of SM-MHC increased by only 10%. Thus, there should be additional factors which modulate SM contractility.

The phosphorylation level of MLC_{20} is a prerequisite of SM contraction initiation and correlates positively with maximal shortening velocity [for review see 22]. Two isoforms of the 20 kDa MLC are expressed in smooth muscle cells of the myometrium designated as MLC_{20A} and MLC_{20B} which are at least mono- and diphosphorylated each [20, 21]. Comparing the phosphorylation patterns of the MLC_{20} of non-hypertrophied with hypertrophied myometrium during maximal Ca^{2+} activation, virtually no change of the relative amounts of the MLC_{20} forms could be observed. In fact, it could be demonstrated that myosin light chain kinase and phosphatase activities were similar in normal and hypertrophied human myometrium [41]. Therefore, increased $Vmax_{app}$ of hypertrophied myometrial fibers cannot be due to elevated phosphorylation levels of the MLC_{20}.

Smooth muscle cells express two alternatively spliced [17, 18] isoforms of the 17 kDa MLC designated as MLC_{17a} and MLC_{17b} with decreasing acidity [24, 34, 40]. MLC_{17a} is also expressed in non-muscle cells while the MLC_{17b} splicing variant is smooth muscle specific [19]. A high expression of the MLC_{17a} isoform could be correlated with high ATPase activity [24] and $Vmax_{app}$ [16, 23]. These results fit quite well to our observations demonstrating an increased MLC_{17a} expression and contractility in hypertrophied myometrial fibers: The relative proportion of the MLC_{17a} isoform increased from 25.5% in normal to 44.2% in hypertrophied human myometrium, i.e., by about 70%. $Vmax_{app}$ increased by a comparable extent, namely 85% during myometrial hypertrophy. This close relation between MLC_{17a} and $Vmax_{app}$ in human is in accordance with our previous study using an animal model: in rat myometrium we demonstrated a significant positive correlation between MLC_{17a} and V_{max} [16].

In summary, the increased contractility of hypertrophied human myometrial fibers correlated positively with an up-regulation of MLC_{17a} expression. Down-regulation of non-muscle MHC expression in favour of SM-MHC as well as the generation of alternatively spliced variants of smooth muscle MHC seem to be of minor relevance for the enhanced contractility of hypertrophied myometrial cells.

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