Isoform Distribution and Tissue Contents of Contractile and Cytoskeletal Proteins in Hypertrophied Smooth Muscle From Rat Portal Vein

Ulf Malmqvist and Anders Arner

Growth of the smooth muscle in the rat portal vein was initiated by an increased transmural pressure. After 7 days, the cross-sectional area of the vessel wall and the maximal active force of the longitudinal muscle layer had increased twofold. Electron microscopy showed that the cell cross-sectional area was increased, suggesting cellular hypertrophy. Increased amounts of intermediate (10 nm) filaments were observed in the hypertrophied cells. The hypertrophied vessels had decreased DNA content per unit wet weight compared with the control vessels (hypertrophied, 1.5±0.1; control, 1.9±0.1 μg/mg; p<0.01). Protein composition was studied with electrophoretic methods. Compared with control preparations the hypertrophied veins had similar myosin and actin contents per unit wet weight (myosin: hypertrophied, 4.4±0.8; control, 5.9±0.9; actin: hypertrophied 12.2±0.6; control, 11.8±1.0 mg/g). Two different forms of the myosin heavy chain were detected with 5% sodium dodecyl sulfate–polyacrylamide gels. The proportion of the lower molecular weight heavy chain relative to total heavy chain content was about 30% and similar in both preparations. The relation filamin/myosin was increased in the hypertrophied vessels. Pyrophosphate gel electrophoresis revealed two protein bands, with an increase in the slower migrating band in the hypertrophied vessels possibly reflecting an increase in filamin content in the extracts. In the control portal vein α-actin is the dominating isoform constituting about 55% of total actin. In hypertrophied vessels, α-actin decreased (by 15%) and γ-actin increased (by 20%). The portal vein contained desmin and vimentin in a ratio of about 6:1. The hypertrophied vessels showed a marked increase in the amount of these proteins (desmin/actin: hypertrophied, 0.32; control, 0.14). In conclusion, during pressure-induced growth of the portal vein, contractile protein contents increase in proportion to the increase in weight. A change in isoforms of actin occurs but no evidence for a change in myosin isoforms was found. The structural proteins increase relative to tissue weight, possibly associated with the increased number of intermediate filaments demonstrated with electron microscopy. (Circulation Research 1990;66:832–845)

The structure of the blood vessel wall shows marked changes during normal development and under conditions of altered pressure load.1 In essential hypertension in humans and in several animal models of hypertension, an increase in arterial wall thickness is found.2 Although the structural changes in hypertension involve most components of the vessel wall, the properties of the smooth muscle cells have a major influence on vessel function and on the hemodynamics and have therefore been extensively studied.

In most types of hypertension, the increase in arterial wall thickness is accompanied with an increased smooth muscle cell mass. However, the specific structural alterations in the vascular smooth muscle cells show a variability that is dependent on such things as the type of vessel studied and the type and duration of the hypertensive state. For aortic smooth muscle cells from spontaneously hypertensive rats, hypertrophy associated with hyperploidy3 or hypertrophy and hyperplasia4 have been reported. In smaller arterial resistance vessels, it is mainly hyperplasia that has been observed.5

In venous vessels, an increased transmural pressure causes hypertrophy of the smooth muscle in the
Although the physiological hemodynamic changes associated with this hypertrophy are not clear, the venous vessels provide an interesting model for study of the hypertrophy process in vascular smooth muscle. A technical advantage is that a large and rapid relative pressure increase can be experimentally introduced without general hemodynamic consequences. Most studies on arterial vessels in hypertension have been performed on large elastic and muscular arteries. The smooth muscle in the rat portal vein, used in the present study, is of the spontaneously active single-unit type and therefore may be similar in some aspects to the smooth muscle in the small precapillary resistance vessels.

A rapid growth of the smooth muscle in the rat portal vein is observed in response to an increased pressure load induced by a partial ligation of the vessel. In this vessel, the growth is considered to be associated with hypertrophy of the smooth muscle cells. After 7 days, the smooth muscle cell mass is doubled. This is accompanied by an increase in the active force generation, suggesting a net synthesis of contractile components in the vessel wall. The mechanical and metabolic properties of the contractile system in the hypertrophied cells are altered. These findings can reflect changes in the excitation-contraction coupling or in the intrinsic properties of the contractile system itself.

The rapid increase in cell size during hypertrophy involves synthesis of cellular proteins. Understanding of the growth process requires information regarding the composition of the newly synthesized proteins. Structural changes, such as the increased amount of intermediate (10-nm) filaments observed in intestinal and vascular smooth muscle, and functional alterations, such as altered force, suggest that quantitative changes in cellular proteins occur in some types of smooth muscle hypertrophy. Also, the possibility that the protein synthesis introduces a shift in the relative amounts of preexisting isoforms or the expression of new isoforms of cellular proteins should be considered. Such qualitative changes may be correlated with altered contractile properties of the cells in a similar manner as described for hypertrophied cardiac muscle. The aim of the present study was to characterize the structural and biochemical properties of the hypertrophying cells in the rat portal vein. Amounts of contractile and structural components were determined with quantitative electrophoretic methods and were correlated with mechanical and structural data. The possibility of a change in the isoforms of myosin and actin was considered.

Materials and Methods

Portal venous hypertension was induced in Sprague-Dawley rats (both sexes, weight 150–200 g) as described by Johansson. The animals were anesthetized with ether, and the two branches of the portal vein entering the liver were partially ligated with 4.0 surgical silk. In sham-operated animals serving as controls, loose loops of silk were placed around the vessel at the same location as in the ligated animals.

After 7 days, the animals were killed by a blow on the neck, and the portal vein was dissected and rinsed in ice-cold Ca2+-free Krebs’ solution with the following millimolar composition: NaCl 122, KCl 4.7, MgCl2 1.2, NaHCO3 15.5, KH2PO4 1.2, glucose 11.5, and EDTA 0.026. The preparation was carefully freed from fat, adventitia, and endothelium, gently blotted between two sheets of filter paper and weighed on a Cahn electrobalance. The tissue was frozen in liquid N2 and stored at −80°C before extraction of myosin by pyrophosphate-containing solution, or homogenization in sodium dodecyl sulfate (SDS) buffer (composition of solutions see below).

Extraction of Native Myosin

Frozen portal veins were crushed at −170°C with a stainless steel mortar. The frozen powder was extracted on ice with a Guba-Straub solution (300 mM NaCl, 100 mM NaH2PO4, 50 mM NaHPO4, 10 mM Na3P2O7, 10 mM EDTA, 1 mM MgCl2, 0.1% NaN4, and 0.1% 2-mercaptoethanol, pH 6.5), during continuous stirring for 30 minutes. After centrifugation at 30,000g at 4°C for 15 minutes, the supernatant was mixed with an equal volume of glycerol and stored at −20°C. In some experiments, the extraction buffer was as described by Persechini et al: 100 mM Na3P2O7, 5 mM EGTA, 1 mM EDTA, 50 mM NaF, 3 mM dithiothreitol (DTT), 10% (vol/vol) glycerol, and 0.1 mM leupeptin, pH 8.8.

Extraction of Dissociated Proteins

Frozen portal veins were homogenized in glass homogenizers at a ratio of 20 mg wet wt/ml of an SDS sample buffer containing 50 mM tris(hydroxymethyl)aminomethane hydrochloride, 1% (wt/vol) SDS, 25 mM DTT, 10% (vol/vol) glycerol, and 0.001% (wt/vol) bromphenol blue, according to Rovner et al. Homogenization was continued until no particulate tissue remained. The homogenate were then boiled for 2 minutes and stored at −20°C for less than 1 week.

Electrophoresis of Native Myosin

Pyrophosphate-polyacrylamide electrophoresis was performed as described by Hoh using an LKB 2197 power supply and a Pharmacia GE-2/4 apparatus (Pharmacia LKB Biotechnology, Bromma, Sweden). The gel rods (5 mm×6 cm) consisted of 4% total acrylamide (3.9% acrylamide, 0.1% bisacrylamide), 20 mM Na3P2O7, and 10% (vol/vol) glycerol. The pH was adjusted to 8.5 after initiation of polymerization. The electrophoresis buffer had the following composition: 20 mM Na3P2O7, 10% (vol/vol) glycerol, 1 mM EDTA, and 0.01% 2-mercaptoethanol, pH 8.5. The buffer was continuously recirculated from the lower to the upper reservoir and cooled to 0±0.5°C. The gels were prerun for 30 minutes at 40 V (constant voltage). After sample application, the gels were run for 24–36 hours at 80
V. The gels were stained overnight with 0.05% (wt/vol) Coomassie brilliant blue R-250 in 10% methanol and 7% acetic acid and then destained with 40% methanol and 10% acetic acid in a Bio-Rad model 556 gel destainer (Bio-Rad, Richmond, California). For photographic reproduction of the gel rods, the gels were photographed when placed in perspex troughs and covered by cylindrical glass rods as described by Le Beau.19

The ATPase activity in the protein bands was revealed by the method of Hoh et al.20 Pyrophosphate was eliminated from the gels by electrophoresis with an ATP-containing buffer in the upper reservoir. The gels were incubated in an ATP solution with Ca++ at 37°C and ATPase activity was detected by the whitish precipitation of calcium phosphate.

The subunit composition of the protein bands on the pyrophosphate gels was analyzed, after the bands were made visible by staining with 0.1% Coomassie brilliant blue G-250 in 3% perchloric acid. Then, the bands were individually cut out with a razor blade, rinsed in buffer (pH 6.5), crushed and taken up in SDS sample buffer, boiled for 2 minutes, and placed on 5% SDS gels. Because the protein content was low, two to three gel bands were pooled and the SDS gels were silver stained as described by Morrissey.21

For determination of the degree of myosin light chain phosphorylation in the pyrophosphate extracts, the samples were mixed with concentrated SDS sample buffer, boiled for 2 minutes, and separated with two-dimensional electrophoresis essentially as described by Driska et al.22 The proteins were separated using Pharmacia 4.5–5.4 amphotolytes in the first dimension (isoelectric focusing) and 13% SDS gels in the second dimension (see below). The degree of phosphorylation (amount of phosphorylated light chain relative to the total amount of 20-kDa light chains) was determined by densitometry of the SDS gels as described by Driska et al.22

SDS-Polyacrylamide Gel Electrophoresis

SDS-polyacrylamide gel electrophoresis was performed as described by Laemmli et al.23 The gels (2×175×140 mm) were prepared with 5% or 7% total acrylamide (made from stock solution containing 30% acrylamide and 0.8% bisacrylamide), 375 mM tris(hydroxymethyl)aminomethane (pH 8.8), and 1% SDS.

Electrophoresis was carried out for 5–7 hours at 60–80 mA (constant current). Each gel was loaded with different amounts of muscle extracts and standard proteins. The gels were stained overnight with 1% (wt/vol) Coomassie blue in 40% methanol and 10% acetic acid and destained with 30% methanol and 10% acetic acid in a Bio-Rad gel destainer until they were clear.

Isoelectric Focusing

The isoelectric focusing gel rods (3 mm × 13 cm) contained 2% Pharmacia 4.0–6.5 amphotolytes, 9 M urea, and 2% Nonidet P-40 (Sigma Chemical, St. Louis, Missouri). After prefocusing for 2 hours, each gel was loaded with 10–30 µl muscle extract. Isoelectric focusing was performed for 18 hours at 500 V and for 1 hour at 1,000 V, with 20 mM NaOH and 10 mM H3PO4 as anode and cathode solutions, respectively. The gels were placed on a 12% SDS gel either directly or after a maximum of 1 week at –20°C. SDS-polyacrylamide gel electrophoresis was performed as described above.

Determination of Protein and DNA Content

Analysis of protein content was performed as described by Lowry et al.24 Muscle samples were homogenized in 1 M NaOH. DNA determinations were made by the method of Labarca and Paigen25 with calf thymus DNA (Sigma Chemical, St. Louis, Missouri) as standard.

Densitometry

The gels were scanned with a GS-300 densitometer (Hoefer Scientific Instruments, San Francisco, California). Identification of the myosin heavy chain, actin, and filamin peaks was made by comigration with purified proteins (from Sigma Chemical) added to the tissue extracts. The intermediate filament proteins desmin (MW 51 kDa) and vimentin (MW 54 kDa) were identified on the 7% SDS gels on the basis of their relative mobility and on the isoelectric focusing gels by their isoelectric points. The protein spots after the second dimension were scanned in both SDS and isoelectric focusing direction and the optical value (A) was calculated as described by Fatigati and Murphy.26

Electron Microscopy

The preparations were fixed by 2% glutaraldehyde in 0.075 M cacodylate buffer (pH 7.4) containing 0.13 M sucrose and 1.2 mM CaCl2. The muscles were fixed either in the animal at the in situ length or in vitro, after a 2-hour equilibration period at 37°C at the optimal length for active force generation.11 The preparations were postfixed in 2% OsO4 at 4°C for 1 hour, bloc stained with uranylacetate, dehydrated, and embedded in epon. After polymerization, transverse or longitudinal sections were cut for light microscopy (thickness 0.5–2 μm) and for electron microscopy (200–600 Å). The relative muscle area in the vessel wall was determined by point counting.

Statistics

Statistical comparisons were made according to Student’s t test for unpaired data. All values are given as mean±SEM, with the number of observations in parentheses.
Hypertrophic Vascular Smooth Muscle

Results

Native Myosin

Since the mobility of myosin on pyrophosphate gels has been shown to be influenced by myosin phosphorylation\textsuperscript{16,27} we used preparations fixed in Ca\textsuperscript{2+}-free solution to obtain myosin in the unphosphorylated state. This was confirmed by determination of the degree of myosin light chain phosphorylation in native myosin extracts from two controls and two hypertrophic veins. The relative amount of the phosphorylated 20-kDa light chains was found to be below the detection level (<5%) in all of these extracts.

Pyrophosphate gel electrophoresis of native myosin extracts from control and hypertrophic veins revealed two closely migrating bands (Figure 1). The faster migrating lower band dominated in control extracts, whereas in extracts from hypertrophic preparations the relative amounts of the bands were about equal. The proportions of the bands were evaluated by comparing the areas on densitometric scans (cf, Figure 1). The mean values for the area of the slower migrating upper band relative to the total area for control and hypertrophic preparations are shown in Figure 2. Compared with the control group, the hypertrophic preparations had a significantly increased amount of the upper band.

The measurement of ATPase activity in the pyrophosphate gels showed activity at the location for the two bands only. However, the calcium phosphate precipitate was not sufficiently demarcated to enable determination whether one or both of the protein bands had enzymatic activity. When the bands were cut out separately and reelectrophoresed on 5\% SDS gels, the slower migrating band contained mainly a 230-kDa protein (filamin) and traces of proteins with MW 200 kDa (myosin heavy chains), whereas the lower band revealed proteins at 200 kDa.

Myosin Heavy Chains and Filamin

As seen in Figure 3, SDS extracts of control and hypertrophic veins exhibit two myosin heavy chains...
and a filamin band in the upper region of 5% SDS gels. The two myosin heavy chain bands (approximate MW 200 and 205 kDa, respectively) were identified on the basis of their molecular weights and by comigration with purified chicken gizzard myosin. We here denote the slower migrating band (SM₁) and the faster (SM₂) according to Rovner et al. Filamin was identified from molecular weight (about 230 kDa) and by coelectrophoresis with purified chicken gizzard filamin.

The relative proportions of SM₁ and SM₂ were evaluated from the SDS gels by densitometry (compare Figure 3). In extracts from both control and hypertrophic preparations the amount of SM₁ was slightly higher than the amount of SM₂ (Figure 3). The data are summarized in the left panel of Figure 4. No significant difference could be observed between the control and hypertrophic groups with regard to the relative proportions of the two myosin heavy chains. For both groups the ratio SM₂/(SM₁+SM₂) was significantly different from 0.5 (p<0.001).

The amount of filamin in relation to the amount of myosin heavy chains (calculated from areas in densitometric scans as F/[SM₁+SM₂]; see Figure 3) was significantly increased in SDS extracts from hypertrophic preparations as shown in the right panel of Figure 4. In comparison with the SDS extracts, higher filamin/myosin heavy chain ratios were found in both groups, when pyrophosphate extracts (Guba-Straub solution) were separated on the SDS gels. The difference with regard to the amount of filamin between the hypertrophic and the control groups was more pronounced when the pyrophosphate extracts were analyzed (Figure 4, right panel).

**Relative Amounts of Contractile and Structural Proteins**

In Figure 5, photographs of SDS-polyacrylamide (7%) gels and corresponding densitometric scans are shown. Filamin, myosin heavy chains, and actin were identified by comigration with standard proteins and are indicated in the figure. Bands with molecular weights of 50–55 kDa corresponding to the intermediate filament proteins desmin and vimentin are seen.

The relative amounts of proteins on the 7% polyacrylamide gels were determined by comparing the areas of the respective peaks on the gel scans. The results are summarized in Table 1. In comparison to the control group the hypertrophic veins had a slightly higher actin/myosin ratio, increased filamin/actin, and increased intermediate filament protein/actin ratios. We also consistently observed a decrease in the relative amount of a protein with an apparent

**Figure 2.** The amount of the slower migrating band (U in Figure 1) on pyrophosphate gels of extracts from control (open bar) and hypertrophic preparations (hatched bar). The amount of the U band is expressed relative to the sum of the areas of the slower (U) and faster (L) migrating bands (see Figure 1 legend). Each portal vein extract was analyzed on three to four gels and the mean value of the data from the gel scans was taken as representative for the preparation. The number of portal vein preparations are given in the figure.

**Figure 3.** Polyacrylamide gels (5%) of SDS extracts from a control (left) and a hypertrophic (right) portal vein. Below each gel, a densitometric scan of the displayed region is shown. F indicates the filamin band and SM₁ and SM₂ the smooth muscle heavy chains with apparent molecular weight of 205 and 200, respectively.
molecular weight of about 90 kDa in the extracts from hypertrophic preparations.

When determining the intermediate filament protein/actin ratios on the 7% acrylamide gels, we did not discriminate between vimentin and desmin since these are not well separated due to the small difference in molecular weight. Thus, the increased intermediate filament protein amount in the hypertrophied preparations may reflect increased amounts of desmin, vimentin, or both. With isoelectric focusing it is possible to separate these proteins. Two-dimensional electrophoretic separation (Figure 6) reveals two isoforms of desmin and two of vimentin. Desmin is the dominating intermediate filament protein in the portal vein. The hypertrophic preparations showed increased amounts of desmin and vimentin. The desmin/actin and vimentin/actin ratio determined in isoelectric focusing gels are shown in the left panel of Figure 7. Thus, the increased amount of intermediate filament proteins in hypertrophic preparations is primarily due to an increase in desmin. The amount of vimentin also increased, and relative to the initial levels of respective protein, vimentin increased more than desmin. The vimentin/desmin ratio was 0.16 in the controls and 0.37 in the hypertrophic vessels.

When actin is separated by isoelectric focusing, three isoforms are revealed: α- , β- , and γ-actin (indicated in Figure 6, compare Whalen et al29), where α-actin is the most acidic isoflorm. With this method, no separation of the two γ-actin forms found in smooth muscle, smooth and nonmuscle γ-actin, can be obtained. Therefore, our estimates of γ-actin include both γ-actin isoforms. The relative proportions of the isoforms determined by scanning of the two-dimensional gels are shown in the right panel of Figure 7. In the portal vein, the α-actin is the dominating form. In the hypertrophic portal vein, γ-actin increased and α-actin decreased.

**Tissue Contents of Actin and Myosin**

Quantitative SDS-polyacrylamide gel electrophoresis was performed to determine the tissue contents of actin and myosin. For each tissue sample, the wet weight was determined before homogenization in SDS sample buffer. Each gel was loaded with three to four different volumes of the sample and four to five different amounts of standard proteins (actin and myosin from chicken gizzard). The area under the protein peaks on the scans and the loaded volume showed linear relations for each sample and corresponding standard. The amount of protein in the sample was calculated from the ratio of the area-volume slope of the sample and the area-protein amount slope of the standard. Determinations of myosin and actin were performed on 5% and 7% polyacrylamide gels, respectively.

As seen in Table 2, the total protein content per unit tissue wet weight in hypertrophic and control preparations was similar. No significant differences were observed in the amounts of actin or myosin per tissue wet weight although the myosin content was slightly lower in the hypertrophic group. All values for myosin relate to the amount of myosin heavy chains. If total myosin is calculated the numbers in

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**Figure 4.** (a) Relative amount of the myosin heavy chains determined in SDS-polyacrylamide (5%) gels. SM1 and SM2 indicate the higher and lower molecular weight heavy chains (see Figure 3). (b) The amount of filament in relation to the content of myosin heavy chains (SM1+SM2) in SDS and pyrophosphate extracts. The open and hatched bars show data from control and hypertrophic preparations, respectively. The number of observations are given in the figure.
Figure 5. Photographs and densitometric scans of 7% polyacrylamide gels. The gels are oriented with the front to the right. The upper and lower panels show separations of proteins from a control and a hypertrophic portal vein, respectively. A protein with an apparent molecular weight of about 90 kDa, which was found to decrease in hypertrophic muscle, is indicated with X.

the table should be multiplied by a factor of 1.2 (assuming heavy chain weights of 203 kDa and light chains of 17 and 20 kDa). DNA content in the tissue was determined with an assay procedure that used a fluorescent drug binding to DNA. The fluorescence was routinely determined about 15 minutes after mixing the assay solution. In three samples from each group, fluorescence was also determined after 60 minutes. Similar fluorescence values were observed, suggesting that no DNase activity was present in the
TABLE I. Myosin Heavy Chains, Actin, Filamin, and Intermediate Filament Proteins in Extracts From Control and Hypertrophic Portal Veins

<table>
<thead>
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<th>Actin/myosin</th>
<th>Filamin/actin</th>
<th>IF/actin</th>
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<tr>
<td>Control</td>
<td>2.3±0.1 (8)</td>
<td>0.08±0.01 (8)</td>
<td>0.16±0.01 (8)</td>
</tr>
<tr>
<td>Hypertrophic</td>
<td>2.8±0.3 (9)</td>
<td>0.11±0.01 (9)</td>
<td>0.37±0.03 (9)</td>
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IF, intermediate filament. Relative amounts were determined by comparing the areas under respective peak on densitometric scans of sodium dodecyl sulfate–polyacrylamide (7%) gels. The amount of myosin refers to the amount of heavy chains only.

homogenate. As shown in Table 2, the hypertrophic veins had a lower DNA content per unit tissue weight compared with the controls.

Morphology

The relative amount of smooth muscle media in preparations, determined on electron microscopy photomicrographs of portal veins fixed in situ, was 62.8±3.0% (n=5) and 67.8±2.0% (n=6) for the control and hypertrophied preparations, respectively. The relative extracellular space in the media was 10% in both groups.11

Electron microscopy photomicrographs of control and hypertrophic preparations are shown in Figure 8. The most notable difference is the marked increase in cell cross-sectional area in the hypertrophied portal veins (compare panels a and b). With higher magnifications thick and thin filaments could be observed in all control and hypertrophic cells. In general, the filaments appeared distributed over the entire cytoplasm. However, in some (less than 1% of the cells in cross-sections) control and hypertrophic cells regular arrangements of thick and thin filaments and regions with only thin filaments could be observed. Panel c in Figure 8 shows a photomicrograph of a region of a control cell with this filament arrangement. A characteristic feature of the hypertrophic cells is the increase in the endoplasmic reticulum (panel b) and an increase in the amount of intermediate (10-nm) filaments (panel d). The intermediate filaments appeared to be localized predominantly in central parts of the cell.

Discussion

The aim of the present study was to characterize the changes in the contractile and structural components in the vascular smooth muscle cells during growth induced by an increase in the vessel wall stress. In the rat portal vein model, used in the present study, the growth was initiated by an abrupt increase in the transmural pressure. The portal vein responds with a growth of the vessel wall within days.11 The time point, used here (7 days) is associated with a doubling of the thickness of the vessel wall and an increased smooth muscle cell mass. The cell cross-sectional area was doubled (Figure 8 present study, compare Malmqvist and Arner11). We presently have no morphometric data regarding the volume of the individual cells. The possibility that the difference in cell cross-sectional area reflects a fixation at different degrees of stretch in the two types of preparations is excluded because the findings were similar in preparations fixed either at the optimal length for force development or at the in situ length. Moreover, in longitudinal sections, no difference in cell lengths of the magnitude needed to explain the increase in cross-sectional area solely on the basis of fixation at different cell lengths was observed. Thus, the morphological data suggest hypertrophy of the smooth muscle cells. A decreased DNA amount per
wet weight (Table 2) is consistent with hypertrophy, assuming unchanged DNA amount per cell. The cross-sectional area at the nuclear level of the cells is doubled with no large change in cell length. If this reflects a doubling of the cell volume, the reduction of DNA/wet weight at unchanged DNA/cell would be higher than the 20% found (Table 2). Thus, the data are consistent with an increased DNA content per cell in this example of smooth muscle hypertrophy. Occurrence of polyploid cells has been reported for some vascular smooth muscle preparations during growth,3 which, if occurring in the hypertrophying portal vein, could explain our findings.

Intermediate filaments are present in most eukaryotic cells. The function of these structures is at present unknown, although they have been proposed to be a component in the cellular cytoskeleton.29,30 In smooth muscle, the intermediate filaments are composed of the proteins vimentin or desmin (compare Lazarides31). The relative proportion of vimentin and desmin varies between smooth muscles from different tissues. We report here that the rat portal vein contains mainly desmin (Figure 6). The desmin/vimentin ratio was 6:1 (Figure 7). In intestinal smooth muscle, desmin has been reported to be the major intermediate filament protein.32 In smooth muscle from rat aorta, however, vimentin is the dominant intermediate filament protein.33 In smaller muscular arteries, the relative amount of desmin increases.34,35 In the rat aorta, two different smooth muscle cell populations, one with mainly vimentin and one with desmin and vimentin, appear to be present.36 At present, there is no information available regarding the intermediate filaments in small resistance vessels. It should be noted that the smooth muscle in the portal vein is of the single-unit type, showing spontaneous contractile activity, and in several aspects it is functionally more similar to the intestinal smooth muscle than to large elastic arteries.

Hypertrophied smooth muscle cells in the rat portal vein have an increased amount of intermediate (10-nm) filaments (Figure 8d and Malmqvist and Arner11). This is in accordance with findings from hypertrophied smooth muscle from guinea pig small intestine14 and rabbit portal vein.7 We can now correlate this structural change with a more than twofold increase in the amount of the proteins desmin and vimentin (Table 1). In the study by Berner et al,7 the calculated mass ratio of intermediate filament to actin was about 2.8 times higher in

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<tr>
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<th>Total protein</th>
<th>Actin</th>
<th>Myosin</th>
<th>DNA</th>
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<tbody>
<tr>
<td>Control</td>
<td>147.2±8.9</td>
<td>11.8±1.0</td>
<td>5.9±0.9</td>
<td>1.9±0.1</td>
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<tr>
<td></td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Hypertrophic</td>
<td>128.7±6.2</td>
<td>12.2±0.6</td>
<td>4.4±0.8</td>
<td>1.5±0.1</td>
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All values are given in mg/g wet weight. The amount of myosin refers to the amount of heavy chains only.

![Diagram](http://circres.ahajournals.org/)

**Figure 7.** Mean values of the relative amounts of desmin and vimentin (a) and the actin isoforms (b). The data were obtained from scans of two-dimensional gels (see Figure 6). The number of observations are given in the figure.
FIGURE 8. Upper panels show electron microscopic micrographs of a control (a) and a hypertrophic (b) portal vein, displayed with the same magnification. Note the increase in cell size and the vacuolar expansion of the endoplasmic reticulum in the hypertrophic cell. Lower panels show higher magnifications of a control (c) and a hypertrophic (d) preparation. The control cell is a representative of cells occasionally found in both control and hypertrophic muscle showing a regular arrangement of thick and thin filaments and regions with only thin filaments. In the hypertrophied cell, an increase (10-nm) in intermediate filaments is observed (arrow).

the hypertrophied rabbit portal vein. In a study of normal rabbit portal veins, Berner et al. using antibodies against vimentin and desmin found that these proteins coexist in the same cell. At present, we cannot determine the cellular distribution of these proteins in the control and hypertrophied portal veins. The major part of the increased amount of intermediate filament protein in the portal vein is desmin. However, relative to the initial amount, vimentin increased more than desmin, giving an
increased vimentin/desmin ratio (0.37 compared with 0.16 in the controls, see Figure 7). In cultured aortic smooth muscle, the cellular amount of vimentin increases and desmin decreases and disappears after five passages.38 Also, during proliferation of the smooth muscle in the intima and media of the rat aorta, following endothelial injury, vimentin increases and desmin decreases or is unchanged.36 Thus, these forms of smooth muscle growth, involving increased number of cells, are associated with increased vimentin. Although the vimentin/desmin ratio increased in the hypertrophied portal vein the major part of the increase in the amount of intermediate filament protein was due to an increase in desmin. This could reflect that desmin is the dominating intermediate filament protein in the normal portal vein. Other possibilities are that the increase in desmin is associated with the type of growth, mainly involving hypertrophy of smooth muscle cells, or with the stimulus initiating the growth.

In all smooth muscle cells, myosin filaments were clearly visible and evenly distributed over the cell cross-section. The thick filaments were often surrounded by a rosette of 10–15 thin filaments. This is similar to the organization of thick and thin filaments described for the rabbit portal vein.39 However, in some cells (less than 1% of cross-sectioned cells) in sections from both control and hypertrophic preparations a more regular filament organization was observed (Figure 8c). These cells showed regions with only thin filaments and regions with regular lattices of thick and thin filaments (about seven to nine thin per thick filament). It seems unlikely that this arrangement is an artifact introduced by the fixation and preparation for electron microscopy since these cells showed a general ultrastructure similar to other cells in the preparation. More likely, this regular filament organization is a property of the living muscle. The regular organization could be present only in some cells or could be a more general filament organization that is lost in most cells during standard fixation.

By using the light and electron microscopy data for the relative muscle area in the preparations together with the relative extracellular space values (controls, 0.11; hypertrophic, 0.14) from Malmqvist and Arner,11 the approximate mean cellular concentrations of actin and myosin heavy chains can be computed for control (actin, 21.2; myosin, 10.5 mg/g cell wet weight) and hypertrophied veins (actin, 20.9; myosin, 7.6 mg/g cell wet weight). These values are lower than those reported by Murphy et al40 for hog carotid artery (actin, 50.3; myosin, 16.2 mg/g cell wet weight). These differences seem to correlate with differences in maximum active force per area (rat portal vein, 130 nN/mm² cell area12; hog carotid artery, 370 nN/mm²40). Comparisons between tissues should however be regarded with caution because factors such as the mode of activation and the assembly and organization of contractile filaments also influence force output. The relation of the amounts of actin and myosin heavy chains in the normal rat portal vein (2.3, Table 1) is in agreement with those previously reported for several other smooth muscle preparations (guinea pig taenia coli, 3.841; hog carotid artery, 3.240; hog arterial, 2.6, and hog nonarterial, 1.542; hog venous, 1.543). In the hypertrophied portal vein, the actin/myosin ratio was slightly, but not significantly, higher than for the controls. This is similar to findings in hypertrophied dog saphenous vein.8

By calculating the number of myosin filaments per cell cross-sectional area on electron photomicrographs, Berner et al7 found a decreased number of thick filaments in hypertrophied rabbit portal vein. These results agree with the decreased force per cross-sectional area reported for other hypertrophic smooth muscle preparations.14 In the hypertrophied portal vein, total active force and the vessel cross-sectional area increases with time.11 After 5 days of pressure increase, active force per area was decreased,10 and after 7 days, the time used in the present study, active force per area was similar to the controls.11 The actin and myosin contents in the hypertrophied portal vein were not significantly different from the values in the controls suggesting that after 7 days, the growing cells have doubled the amount of contractile proteins in the vessel wall. We cannot judge whether this is caused by an increased synthesis rate or a decreased degradation rate of the proteins. The increase in total active force shows that the increased amounts of contractile proteins are organized into functioning filaments. The increase in actin and myosin content in the hypertrophic tissue could be associated with an alteration in the equilibrium between the monomeric and filamentous forms of the contractile proteins. However, this seems less likely since total active force and contractile protein content increase in parallel in the hypertrophic muscle.

It is at present unclear whether smooth muscle myosin can exist as different isoenzymes and if changes in isoenzyme pattern can occur during physiological and pathological smooth muscle growth. An interesting possibility is that the synthesis of contractile proteins during the hypertrophy could cause the formation of different protein isoforms in a similar manner as found for myosin in hypertrophying heart muscle.15 Two isoforms of the myosin heavy chain with approximately equal proportion have been found in several smooth muscle preparations from swine,12 but other ratios have been reported for smooth muscle from other animals.44–46 A third heavy chain has been found in some smooth muscle tissues from humans and in cultured smooth muscle cells.47–49 Recently, it has been shown by complementary DNA cloning and immunoblot analysis using isotype specific antibodies that the smooth muscle myosin heavy chains are encoded by two different messenger RNAs.50 We could not observe any change in the relative amounts of the two myosin heavy chains or an appearance of a third heavy chain in SDS-polyacrylamide gels of extracts from hyper-
trophied rat portal vein. The presence of different isoforms of smooth muscle myosin have been suggested for some muscle tissues on the basis of results from pyrophosphate gel electrophoresis of native myosin.\textsuperscript{51,55} However, the analysis of myosin on pyrophosphate gels is complicated due to the presence of filamin in the bands and the influence of phosphorylation on myosin mobility in the gels.\textsuperscript{16,27,53,54} We find two bands on the pyrophosphate gels and a relative increase in the band with the lower mobility in the hypertrophied rat portal vein. Myosin in our extracts was unphosphorylated and thus the difference was not due to different phosphorylation states of the myosin. The bands show ATPase activity and contain myosin heavy chains. The separation was not sufficient for a determination of ATPase in the individual bands. However, by cutting them out individually, we found that the slower migrating band comprised a protein with a molecular weight of 230 kDa, comigrating with chicken gizzard filamin, and traces of myosin heavy chains. The lower band was comprised of myosin. The increase in the slower migrating band in the hypertrophied muscle could reflect an increase in the ratio filamin/myosin, which is consistent with the increased filamin/myosin ratio found in SDS extracts from hypertrophied veins. This relative increase in filamin is further enhanced in the pyrophosphate extracts (Figure 4). A difference in filamin/myosin ratio between SDS and pyrophosphate extracts has also been reported by Sparrow et al\textsuperscript{54} and could be due to differences in the relative extraction of these proteins in the two media. Since proteins are better solubilized in SDS, the finding could reflect lower extraction of myosin relative to filamin in pyrophosphate. The increased filamin/myosin ratio in hypertrophied muscle could reflect an increase in filamin or a decrease in myosin. The nearly unaltered tissue amount of myosin (Table 2) suggests that the hypertrophied cells contain an increased amount of filamin. This is further supported by the increased filamin/actin ratio and unaltered actin (Tables 1 and 2). The function of filamin in the smooth muscle cell is at present unclear. It has been suggested to be associated with actin and intermediate filaments,\textsuperscript{55} and an increase could be associated with the increase in the intermediate filaments. Thus, the biochemical data in the present study do not give evidence for an isoenzyme difference in the newly synthesized myosin in this case of smooth muscle hypertrophy. Accordingly, the maximal shortening velocity in skinned hypertrophic portal veins under maximal activation has been shown to be unaltered.\textsuperscript{11}

Actin from control and hypertrophied portal veins was separated into three distinct protein spots by two-dimensional electrophoresis. These actin forms, \(\alpha\), \(\beta\), and \(\gamma\), differ in their isoelectric points. \(\alpha\)-Actin, the most acidic form, was found to be the dominating isoform in the portal vein, similar to results from other vascular smooth muscle preparations.\textsuperscript{26} Two types of \(\gamma\)-actin have been demonstrated in smooth muscle. These cannot be separated by two-dimensional electrophoresis,\textsuperscript{56} and the \(\gamma\)-actin protein spot observed in extracts from the rat portal vein could comprise one of the forms or a mixture of both. During development of the rat aorta, the relative amount of \(\alpha\)-actin increases with age.\textsuperscript{57} In contrast, in smooth muscle cell cultures and in proliferating smooth muscle in the intima following endothelial injury the \(\beta\) and \(\gamma\) forms of actin have been found to increase.\textsuperscript{36,58} An increase in \(\gamma\)-actin is observed in human and monkey uterus during pregnancy.\textsuperscript{44} In the hypertrophying portal vein we find an increase in the \(\gamma\), a decrease in \(\alpha\)-actin and unaltered \(\beta\)-actin. The physiological significance of this change in the actin isoforms is unclear at present.

In conclusion, pressure-induced growth of the smooth muscle in the rat portal vein is associated with cellular hypertrophy and ultrastructural changes. Actin and myosin increase in proportion to the increase in smooth muscle volume. These biochemical results correlate with the increased total force and unaltered force per unit muscle area. A change in isoforms of actin is observed. No evidence for a change in myosin isoforms was found. Desmin, vimentin, and filamin increase relative to tissue weight, possibly associated with the increased number of intermediate filaments.

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**References**

Lazarides
Geiger
28. Whalen
27. Lowry
25. Morrissey
15. Persechini
14. Le Beau
Malmqvist U, 10. Malmqvist JFY, 10:1053-1076
Chem 1986;261:6293-6299
Rovner AS, Thompson MM, Murphy RA: Two different heavy smooth muscle chains are found in smooth muscle myosin. Am J Physiol 1986;250:C861-C870
Takano-Omuro H, Kohama K: The mobility of gizzard myosin in pyrophosphate gel electrophoresis is increased by its light chain phosphorylation. J Biochem 1986;100:259–268


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