Contractile and cytoskeletal proteins in smooth muscle during hypertrophy and its reversal

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MALMQVIST, ULF, ANDERS ARNER, AND BENGT UVELIUS. Contractile and cytoskeletal proteins in smooth muscle during hypertrophy and its reversal. Am. J. Physiol. 260 (Cell Physiol. 29): C1085-C1093, 1991.—Hypertrophy of rat urinary bladder smooth muscle was induced by partial urethral obstruction. Bladder weight increased from 70 to 240 mg after 10 days and to 700 mg after 7 wk. Removal of the obstruction after 10 days caused a regression of bladder weight to 130 mg. The relative volume of smooth muscle in the bladder wall increased during hypertrophy. The concentration of myosin in the smooth muscle cells decreased in 10-day hypertrophied bladders, whereas the concentration of actin was unchanged. The actin-myosin ratio was normalized. Two isoforms of myosin heavy chains were identified (SM1 and SM2). The relative amount of SM2 increased in control bladders and decreased in 10-day obstructed bladders. After removal of obstruction, the ratio was normalized. Two isoforms of myosin heavy chains were identified (SM1 and SM2). The relative amount of SM2 decreased during hypertrophy. The relative proportion of actin isoforms (α, β, and γ) was altered toward more γ and less α. These changes were reversible upon removal of the obstruction. Desmin was the dominating intermediate filament protein. The concentration of desmin and filamin increased in the hypertrophic bladders. The increased desmin-actin and filamin-actin ratios in obstructed bladders were normalized after removal of the obstruction. The results suggest that the turnover of contractile and cytoskeletal proteins is fast and can be regulated in response to changes in the functional demands in smooth muscle.

IN GENERAL, SMOOTH MUSCLE CELLS maintain their ability to respond with growth to different types of stimuli after development into the adult phenotype. Increased transmural pressure in arterial and venous vessels initiates smooth muscle growth (8, 21, 44). Also, in other hollow organs such as uterus, intestine, and urinary bladder, increased wall stress and strain can initiate growth (1, 16, 51). Stretch imposed in vitro on smooth muscle tissue has been shown to increase protein content in the preparations (20). Several of these models of smooth muscle growth are associated with cellular hypertrophy or a combination of hypertrophy and hyperplasia. Hypertrophy involves an increase in the mass of several cellular components, e.g., the contractile and cytoskeletal systems and the organelles responsible for protein synthesis (6, 16). Although the increase in muscle mass is accompanied by an increased total-force-generating ability in most models of hypertrophy, force does not necessarily increase in proportion to the muscle mass. For instance, in the rat urinary bladder, hypertrophy is associated with a decreased force per muscle cross-sectional area (4). Similar results have been reported for the rat portal vein 3–5 days after the initiation of growth (49), although in that model the force per area is normalized after 7 days (29). Smooth muscle hypertrophy is associated with alterations in the cellular cytoskeleton with an increase in the (10 nm) intermediate filaments (6, 17, 30). In the 7-day hypertrophied rat portal vein, the unaltered force per area and the increased amounts of intermediate filaments correlate with unchanged cellular amounts of actin and myosin and increased amounts of desmin and vimentin (30). These results indicate that the smooth muscle cell can modulate the cellular amounts of contractile and structural proteins independently during growth.

The smooth muscle mass in the vessel wall can be reduced by reduction of pressure in previously normal arteries (3, 14). The smooth muscle mass is known to decrease during antihypertensive treatment (38) in arterial vessels from hypertensive animals and in uterus during involution after partus (53). Although several aspects of the hypertrophic smooth muscle cells have been investigated during recent years, little is known regarding the properties of the smooth muscle cells during and after regression from a state of increased functional load.

In the present study we have investigated the rat urinary bladder, which exhibits both hyperplasia and hypertrophy of the smooth muscle cells in response to urinary outflow obstruction (60). Strips from such bladders have a decreased force per cross-sectional area 10 days after the initiation of growth (4). The metabolic tension cost, reflecting intrinsic cross-bridge turnover, was unaltered in the hypertrophic muscle (4). The aim of the present study was to investigate if the decreased force per area is accompanied by a change in the amounts and isoform distribution of the contractile and cytoskeletal proteins in this hypertrophied smooth muscle. One of the advantages of this animal model is that the ligature responsible for the outflow obstruction can be removed, thus relieving pressure from the stimulus responsible for the growth (26). This enables the reversibility of the protein changes in the hypertrophied smooth muscle cells to be studied. To relate the protein changes to smooth muscle mass, light and electron microscopy were performed to determine the amount of smooth muscle in the bladder wall for the different preparations used.
Some of the results have been presented in a preliminary form (31, 50).

METHODS

Operative procedure. Female Sprague-Dawley rats weighing 210–220 g were used. Bladder outflow obstruction was induced as described previously (51). The animals were anesthetized with methohexital sodium (Bre-vital), and, via a lower abdominal incision, a ligature of 4–0 surgical silk was tied around the proximal urethra. The degree of obstruction was determined by an indwelling rod (diameter 0.9 mm). Sham-operated animals served as controls. Prior to the operation the animals were selected at random to belong to one of the following groups: 1) 10-day obstructed; 2) 7-wk obstructed; 3) 10-day obstructed, removal of the ligature, and death 7 wk after the initial operation ("unobstructed"); 4) sham-operated controls to 10 day obstructed; 5) sham operated controls to 7-wk obstructed; and 6) controls to group 3, with sham operation 10 days after the initial sham operation.

The operative procedures did not affect the general behavior of the animals and no differences in animal weight or weight gain were observed between the obstructed groups and their controls.

After the observation periods, the animals were killed by cervical fracture and the bladders were quickly taken by cervical fracture and the bladders were quickly taken. Bladder outflow obstruction after the initial sham operation served as controls. Prior to the operation the animals were anesthetized with methohexital sodium (Bre-vital), and, via a lower abdominal incision, a ligature of 4–0 surgical silk was tied around the proximal urethra. The degree of obstruction was determined by an indwelling rod (diameter 0.9 mm). Sham-operated animals served as controls. Prior to the operation the animals were selected at random to belong to one of the following groups: 1) 10-day obstructed; 2) 7-wk obstructed; 3) 10-day obstructed, removal of the ligature, and death 7 wk after the initial operation ("unobstructed"); 4) sham-operated controls to 10 day obstructed; 5) sham operated controls to 7-wk obstructed; and 6) controls to group 3, with sham operation 10 days after the initial sham operation.

The operative procedures did not affect the general behavior of the animals and no differences in animal weight or weight gain were observed between the obstructed groups and their controls.

After the observation periods, the animals were killed by cervical fracture and the bladders were quickly taken out and placed in ice cold Ca2+ free Krebs solution. The bladders were gently blotted between two sheets of filter paper, weighed, frozen in liquid N2, and stored at -80°C before homogenization in sodium dodecyl sulfate (SDS) buffer (for composition, see below).

Extraction of dissociated proteins. Frozen bladders were crushed at -170°C with a stainless steel mortar, and the frozen powder was extracted in glass homogenizers in SDS buffer (20 mg tissue wet wt/ml) until no particulate matter remained. The homogenate was then boiled for 2 min and stored at -80°C. The SDS buffer contained 50 mM tris(hydroxymethyl)aminomethane (Tris) hydrochloride, 1% SDS (wt/vol), 25 mM dithiothreitol, 10% glycerol (vol/vol), and 0.001% bromphenol blue (wt/vol), according to Ref. 41.

SDS-polyacrylamide gel electrophoresis. SDS-polyacrylamide gel electrophoresis was performed as described in Ref. 24. The gels (2 X 175 X 140 mm) were prepared with 5 or 7% total acrylamide (made from stock solution containing 30% acrylamide and 0.8% bisacrylamide), 375 mM Tris hydrochloride (pH 8.8), and 0.1% SDS. Electrophoresis was carried out for 5–7 h at 60–80 mA (constant current). Each gel was loaded with different amounts of muscle extract and standard proteins: chicken gizzard myosin (Sigma Chemical, St. Louis, MO) and actin (Sigma Chemical). The gels were stained overnight with 1% Coo massie blue (wt/vol) in 40% methanol and 10% acetic acid and destained in the same solution without Coo massie blue in a Bio-Rad model 556 gel destainer (Bio-Rad, Richmond, CA) until clarity.

Isoelectric focusing. Two-dimensional electrophoresis was performed essentially as described previously (36). The isoelectric focusing (IEF) gel rods (diameter, 3 mm; length, 13 cm) contained 2% Pharmacia 4.0–6.5 amphotelytes (Pharmacia LKB Biotechnology, Bromma, Sweden), 9 M urea, and 2% Nonidet P-40. After prefocusing for 2 h, each gel was loaded with 5–15 μl of muscle extract. Focusing was performed for 18 h at 500 V. The gels were directly, or after a maximum of 2 wk at -20°C, placed on a 12% SDS gel, and SDS-polyacrylamide gel electrophoresis was then performed as described above.

Densitometry. The gels were scanned with a GS-300 densitometer (Hoefer Scientific Instruments, San Francisco, CA). On IEF gels, the protein spots after the second dimension were scanned in both SDS and IEF dimensions, and the optical value (A) was calculated as A = 3/4 X H X WSIDS X WIEF (13). H is the maximum peak height, and WSDS and WIEF are the widths of the peaks at one-half maximum height in the SDS and IEF dimensions. Identification of the myosin heavy chains and actin and filamin peaks was made by comigration with purified proteins added to the tissue extracts. The intermediate filament proteins desmin (51 kDa) and vimentin (54 kDa) were identified on the 7% gels on the basis of their relative mobility and on the two-dimensional gels by their isoelectric points. In control experiments, we confirmed the identity of these bands on both types of gels with Western blotting analysis using monoclonal antibodies against vimentin and desmin (gift from Dr. J. V. Small). The existence of nonmuscle myosin in the 5% SDS gels was investigated with Western blotting analysis using rabbit antiplatelet myosin (gift from Dr. J. R. ScIers).

Histology. To determine the relative amount of smooth muscle in the urinary bladder wall, bladders filled with 0.7 ml saline were immersion-fixed in 5% glutaraldehyde in 100 mM cacodylate buffer. The bladders were then postfixed in 1% OsO4, for 1 h, dehydrated, and embedded in Epon. For light microscopy, 1- to 3-μm thick sections were cut and stained with azur II methylene blue. For electron microscopy, 600- to 900-Å-thick sections were used. In control experiments, we determined force during fixation in both control and hypertrophic bladder muscles. Force remained unchanged during fixation. Cellular ultrastructure, as judged from the appearance of mitochondria, was normal in both hypertrophic and control preparations. These data seem to exclude major tissue shrinkage or swelling during fixation. Determination of the relative amount of smooth muscle bundles in the bladder wall was made by planimetry. Extracellular volume within the bundles was determined on electron micrographs by point counting.

Statistics. Statistical comparisons were made according to Student's t test. Analysis of variance, with Scheffe's method for determining simultaneous confidence limits, was used when more than two means were compared. All values are means ± SE.

RESULTS

Structural changes associated with outflow obstruction. In the animals with urinary outflow obstruction, the bladder weight had increased threefold after 10 days and ninefold after 7 wk (Table 1). Removal of the obstruction after 10 days caused a partial decrease in the bladder weight toward the control value. The relative amount of
smooth muscle in the bladder wall was increased in the obstructed bladders, and this change was reversed on removal of the obstruction. The relative intracellular space within the bundles was similar in the investigated groups. Within the muscle bundles, smooth muscle cells constitute ~98% of the total number of cells (51). In the present study we found that the cellularity was very low in the connective tissue between the muscle bundles. Thus smooth muscle is by far the dominating cell type in the bladder wall.

### Tissue contents of actin and myosin

A quantitative analysis of the content of actin and myosin in the smooth muscle was made in controls and 10-day obstructed bladders. Linear relations were found between the amount of standard proteins (myosin and actin, 5-40 µg) or the volume of muscle extract loaded on the gel (10-60 µl) and the areas of the investigated proteins on the densitometric scans. The protein content per volume extract was calculated as the ratio of the slopes of the relations between area volume of extract and area amount of standard protein. Determination of myosin and actin was made on 5 and 7% gels, respectively. The amount of myosin is given as the amount of myosin heavy chains only. No. of observations for myosin and actin values included in parentheses. NS, not significant.

**Table 1. Bladder wet weight and smooth muscle content**

<table>
<thead>
<tr>
<th></th>
<th>Control (10 days)</th>
<th>Obstructed (10 days)</th>
<th>Control (7 weeks)</th>
<th>Obstructed (7 weeks)</th>
<th>Control (7 weeks)</th>
<th>Unobstructed (7 weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bladder weight, mg</td>
<td>60±3</td>
<td>238±32</td>
<td>80±2</td>
<td>703±86</td>
<td>79±3</td>
<td>129±7</td>
</tr>
<tr>
<td>Volume of smooth muscle bundles, %</td>
<td>49±3</td>
<td>60±1</td>
<td>51±3</td>
<td>63±4</td>
<td>53±3</td>
<td></td>
</tr>
<tr>
<td>Intracellular space in muscle bundles, %</td>
<td>83±2</td>
<td>85±2</td>
<td>88±1</td>
<td>84±2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. Bladder wet weight (n = 7-9) in control and obstructed animals at different times after operation. Unobstructed group had been obstructed for 10 days followed by removal of ligature, with death 7 wk after initial operation. Relative amount of smooth muscle bundles in bladder wall was determined by light microscopy and relative intracellular space within bundles with electron microscopy (n = 4).

### Relative amounts of actin, myosin, and structural proteins

To get an accurate estimate of the actin-to-myosin ratio and also to compare the amounts of contractile and structural proteins (filamin and intermediate filament proteins), we chose to analyze the relative amounts of these proteins on 7% gels. On these gels, it is possible to get a good separation of filamin, myosin heavy chains, actin, and desmin (Fig. 1). Vimentin was identified with Western blotting as being clearly separated from the desmin band. The most prominent finding in the obstructed group is the increase in the relative amount of filamin and desmin compared with the controls. In Table 3, the summarized data for the relative amounts of the structural and contractile proteins are shown. Hypertrophy for 10 days and 7 wk was associated with an increase in the actin-to-myosin ratio. In the group where the ligature was removed, this value returned toward the control level. In the obstructed group, the filamin-to-actin and desmin-to-actin ratios were almost doubled compared with the control group. Changes in several protein bands were observed in the hypertrophic tissue.

### Table 2. Tissue amounts of myosin and actin in 10 day control and obstructed bladders

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>P</th>
<th>Obstructed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myosin mg/g wet wt</td>
<td>6.9±0.6</td>
<td>NS</td>
<td>5.4±0.7</td>
</tr>
<tr>
<td>(9)</td>
<td></td>
<td></td>
<td>(6)</td>
</tr>
<tr>
<td>mg/g smooth muscle cell wet wt</td>
<td>16.9±1.4</td>
<td>&lt;0.01</td>
<td>10.6±1.3</td>
</tr>
<tr>
<td>(9)</td>
<td></td>
<td></td>
<td>(6)</td>
</tr>
<tr>
<td>µg bladder 450.12±24.4</td>
<td>&lt;0.001</td>
<td>1,403.8±204.9</td>
<td></td>
</tr>
<tr>
<td>Actin mg/g wet wt</td>
<td>17.1±1.9</td>
<td>NS</td>
<td>17.4±1.6</td>
</tr>
<tr>
<td>(7)</td>
<td></td>
<td></td>
<td>(7)</td>
</tr>
<tr>
<td>mg/g smooth muscle cell wet wt</td>
<td>41.7±4.6</td>
<td>NS</td>
<td>34.3±3.5</td>
</tr>
<tr>
<td>(7)</td>
<td></td>
<td></td>
<td>(7)</td>
</tr>
<tr>
<td>µg bladder 1,221.0±124.9</td>
<td>&lt;0.001</td>
<td>3,995.1±698.3</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. Protein concentrations were determined relative to tissue wet wt with quantitative sodium dodecyl sulfate (SDS) gel electrophoresis. Concentration per smooth muscle cell weight was calculated using light and electron microscopy data given in Table 1. Amount of myosin refers to amount of heavy chains only. No. of observations for myosin and actin values included in parentheses. NS, not significant.
FIG. 1. Photograph of 7% sodium dodecyl sulfate (SDS)-polyacrylamide gels of extracts from a control (C, 40 μl), 10-day obstructed (10d O, 60 μl), 7-wk obstructed (7w O, 60 μl), and an initially obstructed bladder where the ligature was removed after 10 days and animals killed 7 wk after initial operation (unobstructed, U, 40 μl). Positions of filamin, myosin heavy chains, desmin, and actin are indicated. Arrowheads with stars denote some protein bands where changes appear during hypertrophy.

(some of these are indicated in Fig. 1). For instance, the relative amount of a protein with an apparent molecular mass of 90 kDa decreased in the obstructed groups.

On 7% SDS gels, the desmin band has been found to include another unidentified protein in some vascular tissues (45). As a further analysis of the intermediate filament proteins, we performed a two-dimensional gel electrophoresis. Figure 2 shows that desmin is the dominating intermediate filament protein in the rat detrusor muscle. The relative amount of desmin to actin, determined by gel scanning on the two-dimensional gels, increases twofold in the ligated bladders (Table 4). No difference in the desmin-to-actin ratio was observed between controls and bladders where the obstruction had been removed. The results regarding desmin compare well with those obtained from the 7% gels (Table 3). Small amounts of vimentin were found on heavily loaded gels and with Western blotting analysis. The vimentin-to-desmin ratio was significantly \( P < 0.05 \) decreased in 10-day hypertrophic muscle \( (0.05 \pm 0.01, n = 4) \) compared with the controls \( (0.10 \pm 0.02, n = 5) \).

Isolforms of actin. Three isoforms of actin were separated with the two-dimensional gel electrophoresis (α, β, and γ in Fig. 2). Note that on these gels, the two forms (Table 3). Relative amounts of contractile and structural proteins

<table>
<thead>
<tr>
<th>Protein</th>
<th>Control (10 days)</th>
<th>Obstructed (7 weeks)</th>
<th>Control (7 weeks)</th>
<th>Obstructed (7 weeks)</th>
<th>Control (7 weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actin/myosin</td>
<td>2.32 ± 0.25</td>
<td>3.29 ± 0.32</td>
<td>2.24 ± 0.14</td>
<td>2.88 ± 0.11</td>
<td>2.41 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>( P &lt; 0.05 )</td>
<td></td>
<td>( P &lt; 0.01 )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Desmin/actin</td>
<td>0.16 ± 0.02</td>
<td>0.31 ± 0.02</td>
<td>0.18 ± 0.02</td>
<td>0.28 ± 0.01</td>
<td>0.19 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>( P &lt; 0.001 )</td>
<td></td>
<td>( P &lt; 0.001 )</td>
<td></td>
<td>( P &lt; 0.01 )</td>
</tr>
<tr>
<td>Filamin/actin</td>
<td>0.09 ± 0.01</td>
<td>0.18 ± 0.03</td>
<td>0.10 ± 0.01</td>
<td>0.16 ± 0.01</td>
<td>0.12 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>( P &lt; 0.05 )</td>
<td></td>
<td>( P &lt; 0.01 )</td>
<td></td>
<td>( P &lt; 0.01 )</td>
</tr>
</tbody>
</table>

Values are means ± SE; NS, not significant. Ratios of amounts of contractile and structural proteins in control, obstructed, and unobstructed bladders determined on densitometric scans of 7% SDS-polyacrylamide gels \( (n = 7-9) \).
organ with dominance of smooth muscle cells and that it is technically possible to remove the stimulus for the hypertrophy. In addition, many of the pathophysiological changes in bladder contractility observed in humans with infravesical outflow obstruction are also present in these animals (28).

The partial ligation of the proximal urethra creates an infravesical urinary outflow obstruction. The cause for the resulting hypertrophy of the wall is unknown; both the distension due to residual urine and the increased work load due to distension (law of Laplace) and resistance to flow may be involved. Smooth muscle is the major cell type in the control and hypertrophied rat detrusor (51, present study). After the ligation, the bladder weight increases threefold in 10 days and fourfold in 7 wk (Table 1). In the hypertrophied bladders, the relative amount of smooth muscle in the bladder wall increased slightly (Table 1). The increase in smooth muscle mass was therefore about 4- and 10-fold for the 10-day and 7-wk groups, respectively. Removal of the ligature after 10 days caused the bladder weight to decrease toward the control value. These findings show that this smooth muscle organ has a great ability to alter its structure in response to changes in the functional load.

Because structural changes during growth may change the relative amount of smooth muscle in the tissue, we considered it important to relate mechanical and biochemical data to the amount of smooth muscle in the bladder wall. In a previous study (4) we determined the active force output in response to various types of stimulation, in 10-day hypertrophic bladders. Force per smooth muscle cross-sectional area had decreased in the hypertrophic muscle. The low force output could be due to several factors, such as alterations in cellular geometry, force transmission, activation processes, amounts and properties of the contractile proteins, and the assembly and organization of the contractile filaments. In the 10-day hypertrophic detrusor muscle, the concentration of actin in the smooth muscle cells was unchanged, whereas myosin had decreased (Table 2). These data are in agreement with a decreased amount of thick filaments as a cause for the decreased force per cross-sectional area in the hypertrophic muscle. A similar mechanism for the decrease in force based on a relative increase in intermediate filaments and a relative decrease in thick filaments was suggested for hypertrophied rabbit portal vein on the basis of electron microscopy (6), although in that study no force measurements were reported. Even if the cellular concentrations of contractile proteins are similar or lower in the hypertrophic muscle, the total amounts in the bladder have increased significantly (Table 2), reflecting a net synthesis of these proteins. At present we cannot discriminate if this is due to an increased synthesis rate of the proteins, a decreased degradation rate, or a combination of both.

The decreased cellular concentration of myosin and unchanged actin in the 10-day hypertrophic bladder results in an increased actin-to-myosin ratio in the hypertrophic muscle. This ratio was also increased in 7-wk hypertrophic bladders. A similar change has been reported for the hypertrophied saphenous vein from the dog (44). Thus, although a net increase in both actin and myosin occurs during growth, the relation between the amounts of the two proteins is not necessarily maintained. In the bladders where the ligature was removed after 10 days, the actin-to-myosin ratio was normalized.

Three isoforms of actin (α, β, and γ) were separated with the two-dimensional gel system. With this method, the two forms of γ-actin in smooth muscle, smooth muscle and nonmuscle actin, cannot be separated. Therefore, our data regarding γ-actin reflect the total amount of both isoforms. In the control rat bladder the amounts of α-actin and γ-actin were about equal. In the hyper-

TABLE 4. Actin isoform distribution and desmin/actin ratios

<table>
<thead>
<tr>
<th></th>
<th>Control (10 days)</th>
<th>Obstructed (10 days)</th>
<th>Control (7 weeks)</th>
<th>Obstructed (7 weeks)</th>
<th>Control (7 weeks)</th>
<th>Unobstructed (7 weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Actin</td>
<td>40.9±2.6</td>
<td>24.3±2.3</td>
<td>43.1±1.7</td>
<td>30.7±1.0</td>
<td>42.0±0.7</td>
<td>35.5±1.0</td>
</tr>
<tr>
<td>β-Actin</td>
<td>18.8±1.1</td>
<td>19.6±2.8</td>
<td>19.6±0.9</td>
<td>16.2±1.0</td>
<td>20.5±1.0</td>
<td>20.3±0.9</td>
</tr>
<tr>
<td>γ-Actin</td>
<td>40.3±2.9</td>
<td>56.3±2.7</td>
<td>37.4±1.0</td>
<td>53.8±1.1</td>
<td>37.9±1.0</td>
<td>44.2±1.1</td>
</tr>
<tr>
<td>Desmin/actin</td>
<td>0.21±0.03</td>
<td>0.40±0.04</td>
<td>0.14±0.02</td>
<td>0.21±0.02</td>
<td>0.12±0.01</td>
<td>0.12±0.02</td>
</tr>
</tbody>
</table>

Values are means ± SE; NS, not significant. Relative amounts of actin isoforms and desmin/actin ratios in control, obstructed, and unobstructed bladders determined by densitometric scanning of second dimension SDS-polyacrylamide gels after isoelectric focusing (n = 6).
trophic muscles α-actin decreased and γ-actin increased. In hypertrophied smooth muscle from the rat portal vein, a small change in the actin isoforms in the same direction was observed (30). A decrease in α-actin, with an increase in β-actin, is observed in proliferating smooth muscle in tissue culture and in aorta after endothelial denudation (23, 39, 45). This change in actin in smooth muscle seems to be characteristic for the transition from contractile to synthetic phenotype (11). The ratio between total actin and myosin in the hypertrophic muscle increased (Table 3). Due to the change in the relative amounts of actin isoforms (Table 4), the relations between myosin and the different actin isoforms are changed, e.g., α-actin/myosin decreased in the 10-day hypertrophic muscle from the control value of 0.94 to 0.79. In the 7-wk hypertrophic muscles and in the unobstructed group, the ratio was 0.90 and 0.91, respectively. It is possible that these changes in actin isoform pattern are related to the decreased isometric force in the 10-day hypertrophied muscles in addition to an effect of a decrease in myosin as discussed above. However, in solution studies, the different actin isoforms appear to have similar effects on actomyosin adenosinetriphosphatase (34). Little is presently known regarding the influence of the different actin isoforms on actin-myosin interaction in the organized contractile system of the muscle cells. The cellular actin has been suggested to be distributed into a contractile domain containing myosin and a cytoskeletal domain with intermediate filaments and filamin (15, 46). The distribution of the different actin isoforms between the two domains and possible changes induced by the hypertrophy process are at present unknown.

Two myosin heavy chains with apparent molecular masses of ~200 and 205 kDa were found in the rat bladder smooth muscle. Two myosin heavy chains, in equal proportions, have previously been reported in other adult mammalian smooth muscle tissues (41). Unequal proportions, with ~40% SM2, have been reported for the rat uterus (48), rat portal vein (30), and rat aorta (19, 22). As discussed by Sparrow et al. (48), these findings seem to exclude that the myosin molecule necessarily exists as a heterodimer of SM1 and SM2 in these smooth muscle types. Both in the control and in the hypertrophied bladders, the higher-molecular-weight heavy chain (SM1) was found in higher concentration (Fig. 3). The proportion between the heavy chains is similar to those in the other rat smooth muscle tissues mentioned above. Thus it seems that the proportion between the heavy chains does not vary between tissues in the rat. However, the relative amount of SM2 seems to vary between species: guinea pig taenia coli, 55% (41); hog carotid artery and trachea, 50% (33, 41); turkey gizzard, 62% (22); rabbit aorta, 45% (41) and 47% (7); and human bladder, 60% (50).

In the rabbit urinary bladder (32) and aorta (7) and in the hog trachea (33), the proportion of the heavy chains has been reported to change with age of the animal. A third heavy chain has been reported to be expressed in cultured smooth muscle cells (22, 40) and in the pulmonary artery from humans (43). We report here that in the hypertrophied bladders, 10 days and 7 wk after onset of obstruction, a small but significant decrease in the lower molecular weight heavy chain (SM2) was found (Fig. 3). Preliminary results from hypertrophied human bladder (50) suggest an alteration toward increased amounts of SM2 in this form of growth. The shift in the heavy chain isoforms in the rat bladder is reversible because the normal proportions were regained in bladders where the ligature was removed. We have not found evidence for a third heavy chain of the nonmuscle type, and the change in the heavy chains in the hypertrophic detrusor could thus reflect a shift in the amounts of heavy chains expressed in normal tissue. The two heavy chains in smooth muscle are considered to be encoded by one gene and formed by alternative RNA splicing (35). The difference in the amounts of the two heavy chains could thus reflect a change in the regulation at this level. Further characterization of the processes during hypertrophy and regression of the hypertrophic state requires an analysis of tissue mRNA. The intrinsic turnover of cross bridges in muscle cells is considered to be reflected in mechanical parameters (e.g., maximal shortening velocity, V_max) and the energetics of contraction [e.g., metabolic tension cost; cf. review by Ruegg (42)]. In a previous study, we found similar metabolic tension cost using two modes of activation in control and 10-day hypertrophied rat urinary bladders (4). These data suggest that the shift in heavy chain pattern in the hypertrophic muscles is not associated with major changes in cross-bridge kinetics. Also, when different muscle tissues are compared, the heavy chain isoform pattern does not correlate with the maximal shortening velocity and metabolic tension cost. The guinea pig taenia coli and the rat portal vein have about the same V_max [taenia, 0.35 L/s at 37°C (27); portal vein, 0.4 L/s (29)] but differ with regard to the ratio SM2/(SM1+SM2) [taenia, 55% (41); portal vein, 30% (30)]. Conversely, the rat portal vein
and the rat aorta have about the same SM2/(SM1+SM2) ratio (30-10%; Refs. 19, 22, 30) but differ with regard to both V_{max} [portal vein 0.41/s (29); aorta, 0.056/s (5)] and metabolic tension cost (2).

In smooth muscle the intermediate filaments are composed of desmin or vimentin (25). The relative proportion of the two proteins varies between smooth muscle tissues. At present the function of the intermediate filaments is largely unknown (18), although these structures have been proposed to constitute a part of the cellular skeleton in smooth muscle (47). In the normal rat detrusor muscle, desmin is the dominating intermediate filament protein with small amounts of vimentin, giving a vimentin-to-desmin ratio of 0.05. This predominance of desmin is also found in other types of intestinal smooth muscle (9) and vascular tissue from small muscular arteries (37) and in the rat portal vein (30).

The hypertrophied detrusor muscle doubled its amount of desmin after 10 days. As judged from the desmin-to-actin ratio the increased amount of desmin is also present after 7 wk. An increase in intermediate filament associated proteins has also been reported in the hypertrophied portal vein of the rat (30). These biochemical findings correlate with the structural observations that the number of intermediate filaments increases in hypertrophied smooth muscle (6, 17, 30). In the hypertrophied detrusor smooth muscle the vimentin-to-desmin ratio decreased. Although desmin is the dominating intermediate filament protein in the hypertrophied tissue, there is also an absolute increase in vimentin content in these bladders. The changes in intermediate filament associated proteins in the hypertrophying rat bladder and portal vein (30) differ from those observed in proliferating aortic smooth muscle cells both in vivo and in vitro (10, 39) where desmin decreases and vimentin increases. This could reflect a difference in the stimuli leading to growth or that desmin is the dominating intermediate filament protein in the rat detrusor, whereas vimentin is the dominating intermediate filament protein in the rat aorta and in the isolated cells for tissue culture. Another cause could be that, in the latter types of growth, the smooth muscle phenotype may change toward a more synthetic (noncontractile) type (11). In the hypertrophying rat bladder, growth is associated with both hyperplasia and hypertrophy of the cells (51). It is possible that the decrease in active force per muscle area is associated with a change in the phenotype toward the synthetic form in some cells, but because total smooth muscle mass increases fourfold and force per muscle area decreases by only ~50%, a large fraction of the cells are of the contractile type. The function of the intermediate filaments could be related to the growth process. This is less likely, however, because the 7-wk hypertrophied bladders still have an increased concentration of desmin, whereas the growth process has almost subsided (51). Another possibility is that the intermediate filaments have a mechanical function that necessitates an increased concentration in the larger hypertrophied cells. In the unobstructed bladders, cell size is normalized (26). This is associated with a normalization of the amount of desmin, showing that the hypertrophy associated changes in structural proteins are reversible.

An increase in the filamin-to-actin ratio is observed in the hypertrophied muscles. The unaltered concentration of actin indicates a net increase in filamin in the hypertrophied cells after 10 days. Interestingly, the filamin-intermediate filament protein ratio is similar for the controls, hypertrophied, and unobstructed bladders. Thus the increase in filamin could be associated with the increase in cytoskeletal structures. According to structural data, filamin is considered to be associated with the intermediate filaments and actin (46). The function of filamin in the cell is at present unknown, but, if it is associated with structural components, effects on contractile function might be less plausible. In vitro, filamin has been shown to inhibit actomyosin adenosine triphosphatase at a low concentration and potentiate in high (12). In the hypertrophic muscles, the force per muscle area is decreased in association with a decrease in myosin concentration as discussed above. As the metabolic tension cost was unchanged in the hypertrophic muscle (4), despite the increased filamin concentration, a major influence of filamin on the intrinsic rate of cross-bridge interaction is less likely.

Our electrophoretic analyses reveal changes in several protein bands. In the present study we have investigated the contractile and structural proteins. Several unidentified proteins also change (cf. Fig. 1); e.g., a protein with a 90-kDa molecular mass decreases in amount. Further work is required to identify these proteins and their function in the hypertrophic smooth muscle cell.

We show in the present study that pronounced changes in both contractile and cytoskeletal proteins occur in growing smooth muscle in vivo within a time scale of days. These changes are still present after several weeks of growth. The total amount of contractile proteins increases during the growth, with changes in isoform pattern of myosin heavy chain and actin occurring. The changes in isoform distribution of the contractile proteins appear not to be associated with changes in the properties of the actin-myosin interaction in the organized contractile system as judged by the unaltered metabolic tension cost (4). The decrease in force per cross-sectional muscle area (4) is correlated with decreased concentration of myosin in the hypertrophied smooth muscle cells. The rapid changes in the amounts of contractile and cytoskeletal proteins during development and regression of hypertrophy suggest that the turnover of these proteins is fast and can be regulated in response to changes in the functional demands, although the intracellular signal responsible for the regulation in smooth muscle is at present unknown.

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