Force-Generating Capacity and Contractile Protein Content of Arterial Smooth Muscle

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ABSTRACT After correction for extracellular space (40%) determined from electron micrographs, the maximum isometric force developed by strips prepared from the media of the hog carotid artery (2.2×10^6 dyn/cm^2) can be extrapolated to give a value of 3.7×10^6 dyn/cm^2 for the smooth muscle component of the strip. Three independent estimates of the myosin content of the smooth muscle cells were made based on (*a*) exhaustive extraction and purification with estimates of preparative losses, (*b*) the myosin catalyzed ATPase activity of media homogenates, and (*c*) quantitative densitometry of the peaks containing myosin, actin, and tropomyosin after disk electrophoresis of sodium dodecyl sulfate-treated media homogenates. The results were consistent and gave a myosin content of 5–10 mg/g media, or 8–17 mg/g cell. Method (*c*) gave myosin:actin:tropomyosin weight ratios of 1:3.2:0.8. Although measured force developed by the smooth muscle cell exceeds that of mammalian striated muscle, the myosin content in smooth muscle is about five times lower. The actin content of smooth muscle is relatively high. The actin and myosin contents are consistent with thick and thin filament ratios observed in electron micrographs of vascular smooth muscle.

INTRODUCTION

The maximum isometric force that is developed by a muscle at its optimum length is a common measurement that reflects the interaction of the contractile proteins. However, a spectrum of anatomical variations in muscle size and type make this measurement unsuitable for the comparison of different muscles. "Intrinsic strength" may be defined (Close, 1972) as force developed as a function of the amount of contractile material (or the number of cross bridges) acting in parallel within the muscle, and is a desirable measure of contractile function. Since an estimate of the intrinsic strength requires information not presently available, the commonly used comparative index is force developed/cross-sectional area of the muscle. The use of this index assumes: (*a*) that maximum force is elicited and is measured in the axis of force development; (*b*) that extracellular spaces are comparable; and (*c*) that myofilament length and packing are similar.

For the special case of mammalian skeletal muscles, the first condition can be met with proper experimental procedures and the second two assumptions are approximately fulfilled. The best values for maximum isometric force development in mammalian skeletal muscle (reviewed by Close, 1972) are in the range of 1.5–3.0 $\times$ 10^6 dyn/cm^2. This consistency implies a fairly constant intrinsic strength in a variety of muscles which differ greatly in contraction velocities. Published values for mammalian smooth muscle (reviewed by Herlihy and Murphy, 1973 and 1974) range from 0.13 to 2.2 $\times$ 10^6 dyn/cm^2. However, because the three assumptions outlined above cannot generally be validated, the figures do not imply that the intrinsic strength of the smooth muscle contractile system averages less than that of striated muscle, nor that the force-generating capacity of smooth muscles varies widely. This study of the media of the hog carotid artery was undertaken to evaluate the force-generating capacity of a smooth muscle under conditions allowing a more general comparison with other muscle types.

METHODS

Smooth Muscle Preparation

Common carotid arteries obtained from 80–90-kg hogs at slaughter were slit longitudinally and narrow strips of the media were teased out yielding segments of longitudinally oriented smooth muscle cells free of adventitial connective tissue. Details of the preparation have been presented (Herlihy and Murphy, 1973). The strips had cross sections of 0.4–0.8 mm^2 and were approximately 1 cm long when stretched to their optimum length for tension development. The preparation was maintained in a physiological salt solution containing (mM): NaCl, 119.0; KCl, 4.7; KH_2PO_4, 1.18; $MgSO_4$, 1.17; $NaHCO_3$, 14.9; $CaCl_2$, 1.6; D-glucose, 5.5; and EDTA, 0.026, and bubbled with 95% O_2–5% CO_2 (pH 7.4 at 37 C). Maximum active tension was elicited by equimolar substitution of KCl for NaCl and an increase in [$CaCl_2$] to 5 mM (Herlihy and Murphy, 1973). Strips were also prepared from the carotid arteries of a series of weanling hogs (10–20 kg) after sacrifice in the laboratory. This procedure was designed to detect deleterious effects of the slaughter and transport processes which might occur with material obtained from a packing house, and to determine if such strips had a lower connective tissue content.

Anatomical Measurements

Because the strips possessed a relatively constant cross section, the cross-sectional area could be estimated by dividing the weight of the lightly blotted strips by their length and density (taken as 1.05 g/cm^3). These estimates had a high correlation with direct optical measurements (Herlihy and Murphy, 1973). The ratio of media (plus a very thin intimal layer) to artery wet weight was obtained by carefully stripping off successive layers of adventitial tissue from arterial segments until a tube of the muscle-containing medial layers remained. This procedure is feasible because of the easy separation of the various layers in the hog carotid artery. The fractional cell cross-sectional areas were obtained by weighing cutouts of cells from enlargements of

low-power electron micrographs of random fields in the strips. The tissue was fixed in 2% isosmotic glutaraldehyde, washed in cacodylate buffer, postfixed with osmium tetroxide, and embedded in Epon. Sections were stained with uranyl acetate and lead citrate and examined at magnifications not greater than × 1,800 using a Zeiss EM9A electron microscope (Carl Zeiss, Inc., New York, N. Y.).

In Vitro Mechanical Measurements

Determination of passive elastic properties and maximum active isometric tension are difficult in vascular smooth muscle due to the presence of tone, hysteresis effects, and variable activation of the contractile system. Procedures to determine the passive and maximum active length-tension relationships as well as the apparatus used have been described (Herlihy and Murphy, 1973). After a prolonged equilibration period when contractile responses were stable, a partial length-tension diagram was obtained. Maximum isometric tension (P_o) at the optimum strip length was obtained as the difference between the potassium-induced tension developed and the passive elastic tension obtained from quick-release experiments.

Contractile Protein Preparations

Myosin cannot be differentially extracted from smooth muscle due to the high solubility of the actomyosin complex and a variety of associated proteins. Consequently actomyosin was extracted from carotid arteries and purified to stage II (Murphy, 1971) and dissolved in 0.6 M KCl, 40 mM pyrophosphate (pH 8.0), 5 mM ATP and 1 mM EGTA. This solution was centrifuged 5 h at 50,000 rpm in the Beckman 60 Ti rotor (Beckman Instruments, Inc., Fullerton, Calif.) (178,000 *g* average force) to sediment actin, undissociated actomyosin, or aggregated myosin. One-half to two-thirds of the total myosin is sedimented and lost in this procedure. The myosin in the supernatant can be selectively precipitated by adding polyethylene glycol-6000 (J. T. Baker Chemical Co., Phillipsburg, N. J.) as a 20% (wt/vol) solution in 0.2 M KCl to give a final concentration of 2 g/100 ml. After stirring for 20 min the precipitated myosin is collected by centrifugation (10,000 *g*), washed twice with 50 mM KCl, and dissolved in 0.6 M KCl. The myosin shows a high degree of purity (over 80%) on disk electrophoresis.

Actin could be obtained in small amounts from the tip of the ultracentrifuge pellet or extracted from an acetone-dried arterial powder and purified according to procedures developed for skeletal muscle (Spudich and Watt, 1971). Tropomyosin was obtained from the acetone-dried powder after actin extraction (Spudich and Watt, 1971). An attempt to isolate a smooth muscle troponin by methods developed for skeletal muscle (Hartshorne and Mueller, 1969) was unsuccessful.

A variety of biochemical measurements was performed on a "media homogenate" preparation. Tubes containing only the media (plus the thin intimal layer) were prepared as described above and washed in cold 0.3 M sucrose. They were frozen in liquid nitrogen and crushed to a powder in a stainless steel percussion mortar precooled in liquid nitrogen. The powder was suspended in 0.55 M KCl (24 mg/ml) and homogenized with a Polytron blender (Brinkmann Instruments, Inc., Westbury, N.Y.). Protein concentrations were determined by micro-Kjeldahl methods assuming

a 16% protein nitrogen content, or by the biuret technique (absorbance measured at 540 nm) if nonprotein nitrogen was present.

ATPase Activity

Although the Mg^{++}-activated actomyosin ATPase activity of smooth muscle at an ionic strength of 0.1 is very low, the Ca^{++}-activated ATPase activity of myosin isolated from hog carotid arteries is comparable to that of myosin obtained from slow skeletal muscle of larger animals at an ionic strength of 0.6. Consequently, ATPase activity was assayed under the following conditions: 0.1 mg myosin/ml or 16 mg media homogenate/ml, 18 mM morpholinopropane sulfonic acid (MOPS) (pH 7.0 at 25°C, buffering value = −10 mM H^+/pH unit), 5 mM $CaCl_2$, 5 mM ATP, and 0.55 M KCl. Some assays contained 5 mM sodium azide as an inhibitor of mitochondrial ATPase (Fanburg and Gergely, 1965), 0.1 mM ouabain as an inhibitor of Na^+-K^+ ATPase (Glynn, 1968), and 2 mM sodium desoxycholate as a general inhibitor of membrane ATPases. In some assays the media homogenate was incubated with 0.2 g of Dowex 50-X8/ml homogenate suspension for 60 min to remove traces of Mg ions which might activate mitochondrial, microsomal, Na^+-K^+, or actomyosin ATPases. The Dowex beads were removed before determination of the homogenate ATPase activity. As myosin is unusual in having a fairly high ATPase activity in the absence of all divalent cations, the activity of pure myosin or media homogenates was also determined in the presence of 2 mM EDTA in place of the usual 5 mM Ca^{++}. All reactions were run as time-courses after initiation by ATP addition. Reactions were stopped by the addition of equal volumes of cold 5% (wt/vol) TCA. After filtration to remove precipitated protein, ATP hydrolysis was estimated by determination of inorganic phosphate using the method of Rockstein and Herron (1951).

Disk Electrophoresis

The purity of myosin preparations and the contractile protein content of the media preparation were measured by electrophoresis of sodium dodecyl sulfate (SDS)-treated proteins on 7 or 10% acrylamide gels. Protein (0.5 mg/ml) was incubated at 100°C for 1 h in 1% (wt/vol) SDS, 1%, (vol/vol) β-mercaptoethanol, and 10 mM sodium phosphate buffer (pH 7). In certain experiments (Results) the incubation mixture also contained 8 M urea or 0.1 M Na_2SO_3. From 5 to 35 μg of the SDS-treated protein were applied to the gels prepared as described by Weber and Osborn (1969) and subjected to an electrophoresis current of 5 mA/tube. Gels were stained for 2 h in 0.25% Coomassie blue, 45% methanol, and 10% glacial acetic acid and destained by diffusion in 7.5% acetic acid and 25% methanol. Densitometry of the gels was performed on a Gilford spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, Ohio) at 620 nm to estimate protein content in each peak (Lowey and Risby, 1971). Peak areas were determined with a planimeter.

RESULTS

Force Development/Cell Cross-sectional Area

The maximally stimulated arterial strips developed an active force (2.2×10^6 dyn/cm^2) comparable to that of skeletal muscle, although only 60% of

the strip cross section was occupied by smooth muscle cells (Fig. 1). Correction for extracellular space yields a calculated force development of 3.7×10^6 dyn/cm^2 for the smooth muscle. The carotid media of weanling hogs was relatively more fibrous and the active force per cell cross-sectional area developed in response to potassium stimulation was slightly lower (Fig. 1). This may be a physiological difference reflecting less effective activation of the contractile system, a lower contractile protein content, etc.; or it may artifactually reflect relatively greater cell damage in the smaller strips (edge effects).

Estimation of Myosin Content from ATPase Activity Measurements

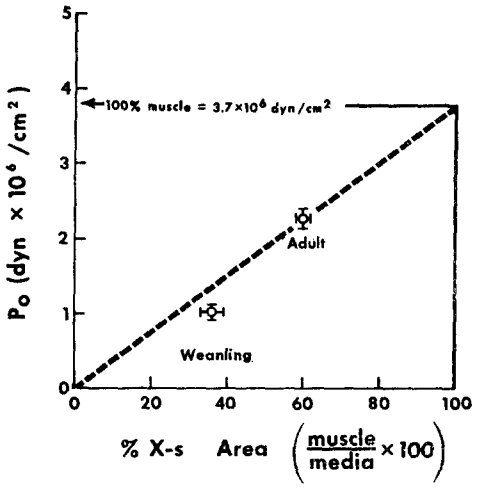
In general, the enzyme content of a tissue can be estimated as:

$$\text{Content}\left(\frac{\text{mg enzyme}}{\text{g tissue}}\right) = \frac{\text{homogenate activity}\left(\frac{\text{mol substrate}}{\text{g tissue}\cdot\text{min}}\right)}{\text{enzyme specific activity}\left(\frac{\text{mol substrate}}{\text{mg enzyme}\cdot\text{min}}\right)}.$$

In practice, two conditions must be fulfilled for this approach to be valid: (*a*) homogenate activity is not altered by inhibiting or potentiating substances; and (*b*) the reaction measured is not catalyzed by other enzymes under the conditions used.

The first assumption was tested by adding pure arterial myosin in varying concentrations to a constant amount of arterial homogenate (Fig. 2). If inhibitors (or activators) were present in the homogenate they would tend to cause deviations from linearity as their concentration relative to the added myosin was altered. It is apparent (Fig. 2) that the relationship was linear. Furthermore, the slope of the line in Fig. 2 gives the specific activity (μmol P_i/mg myosin·min) of myosin in the presence of homogenate and this was equal to that of the myosin preparation when tested in the absence of homogenate. In this respect, vascular smooth muscle resembles skeletal (Sréter et al., 1966; Maddox and Perry, 1966) and cardiac (Vierling et al., 1968) muscle where no evidence for tissue substances which alter myosin ATPase activity has been found.

The second of the two conditions (above) can only be met by making the enzymatic measurement under conditions which minimize ATP hydrolysis by ATPases of mitochondrial, microsomal, plasma membrane, and perhaps other origins. Azide, ouabain, and the Dowex 50 pretreatment had relatively modest inhibitory actions suggesting that under the test conditions most of the ATP hydrolysis was by a Ca^{++}-activated ATPase. Desoxycholate was found in control experiments to partially inhibit pure myosin activity, accounting for the larger inhibitory effect in the reactions containing this detergent. The ATPase activity of the media homogenates and pure arterial



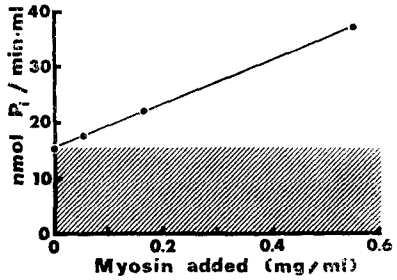


FIGURE 1

FIGURE 2

FIGURE 1. Relationship between force developed in media strips teased from the carotid arteries of 11 adult (80–100 kg) and 11 weanling (12–20 kg) hogs and the percent cross-sectional area of the strip occupied by smooth muscle cells in 15 (adult) and 4 (weanling) fields sampled by electron microscopy. Bars represent ±1 SEM for force (vertical) and area (horizontal) measurements. The dashed line illustrates the graphical determination of force developed per square centimeter muscle cells in adult animals.

FIGURE 2. ATP hydrolysis rate as a function of the concentration of purified myosin measured in the presence of 16 mg homogenized carotid media per milliliter reaction solution. Shaded area corresponds to level of ATP hydrolysis by the media homogenate alone. Conditions: 550 mM KCl, 18 mM MOPS buffer (pH 7.0 at 25°C), 5 mM $CaCl_2$, and 5 mM ATP. The slope of the line gives the specific activity of myosin in the presence of media (44 nmol P_i/mg·min) and was equal to the activity of the myosin in the absence of media homogenate.

myosin using (*a*) the Dowex pretreatment, (*b*) a combination of Dowex, azide, ouabain, and desoxycholate, and (*c*) EDTA was studied more extensively to give the data required to estimate the myosin content of the media (Table I). The values for myosin content of the arterial media obtained would be high in the absence of inhibitors. The remaining estimates (Table I) vary from 4.5 to 9.2 mg myosin/g media, a range which may reflect both the experimental errors and the possible contributions of ATP hydrolysis by other enzymes.

Estimation of Media Contractile Protein Content by Densitometry of SDS Gels

Densitometry of stained SDS gels has been successfully applied to the determination of the relative amounts and stoichiometry of proteins or their subunits in fairly simple systems (cf. Lowey and Risby, 1971; Tregear and Squire, 1973). This technique could also be used to obtain maximum estimates for the contents of a few proteins which constituted the majority

TABLE I

ESTIMATES OF CAROTID MEDIA MYOSIN CONTENT FROM ATPASE ACTIVITY MEASUREMENTS

Preparation (*N*)	Units	Conditions: Activator.... Ca^{++} / Inhibitor.... — — —	Ca^{++} / Dow	Ca^{++} / Dow, Az Oua, Des	K^+ / EDTA
I. Media homogenate (3)	$\frac{\mu mol\ P_i}{g\ media \cdot min}$*	0.97 ±0.05	0.69 ±0.19	0.27 ±0.01	0.30 ±0.11
II. Purified myosin (4)	$\frac{\mu mol\ P_i}{mg\ myosin \cdot min}$*	0.075 ±0.019	(0.075)	0.031 ±0.012	0.067 ±0.023
III. Estimated myosin content	$\frac{mg\ myosin}{g\ media}$‡	12.9 ±0.9	9.2 ±1.1	8.7 ±1.2	4.5 ±1.1

* Values = means ±1 SEM

‡ Values = quotient ($M_{III} = M_I/M_{II}$) ± precision index calculated as $[M_{III}(SEM_I^2/M_I^2 + SEM_{II}^2/M_{II}^2)]^{0.5}$, where SEM_I = standard error for mean values (M_I) of the media ATPase, and SEM_{II} = standard error for mean values (M_{II}) of myosin ATPase.

of the total protein in complex mixtures (Sender, 1971; Kendrick-Jones, 1973). The contractile proteins of muscle would represent such a system since myosin and actin represent about half the total protein in skeletal muscle and the other major constituent, collagen, does not enter the SDS gels. Application of this method requires (*a*) identification of the bands in SDS gels of media homogenates which contain the individual contractile proteins, (*b*) determination of the direct proportionality between area under the peaks of the densitometer tracing and the amount of protein in each band, (*c*) showing that unknown proteins do not constitute a significant fraction of the protein in the bands of interest, and (*d*) demonstration that the SDS treatment effectively reduces all proteins to their constituent polypeptides migrating as single bands.

Electrophoresis of SDS-treated carotid media homogenates yields a small number of bands with high protein concentrations (Fig. 3). The bands containing myosin heavy chains, actin, and tropomyosin were identified by the addition of the purified proteins (Fig. 3). Estimated subunit molecular weights based on mobility in SDS gels (Weber and Osborn, 1969) and comigration with purified proteins from skeletal muscle were 200,000 for myosin heavy chains, 45,000 for actin, and 36,000 to 38,000 daltons for the tropomyosin subunits. Purified tropomyosin preparations showed evidence of symmetrical splitting (as observed by Sparrow and Bockxmeer, 1972), suggesting that there are two subunits of similar molecular weight corresponding to those from skeletal muscle tropomyosin. Our preparative methods yielded no

arterial protein resembling skeletal troponin, and the gels of media homogenates showed no evidence of subunits corresponding to those of skeletal muscle troponin in amounts stoichiometrically related to tropomyosin. Fig. 4 shows that calibration curves run with each set of gels were linear so that accurate quantification could be obtained. Both arterial myosin and actin were used in these calibrations and each yielded the same protein content estimates.

There is no direct test of the assumption that the bands containing myosin, actin, and tropomyosin are not significantly contaminated with other proteins. Consequently, all protein estimates are maximal values. However, in the presence of urea the tropomyosin subunits from skeletal muscle migrate more slowly in SDS gels and appear to have molecular weights around 52,000–54,000 daltons (Sender, 1971). We found arterial tropomyosin was affected in the same way by urea. Consequently, arterial homogenates were treated with urea to shift the tropomyosin migration pattern to a region of the gel having no major peak. Media homogenates incubated in 8 M urea for 2 h before SDS treatment showed a shift of 49.5% of the protein in the peak shown to contain tropomyosin to a location corresponding to an apparent

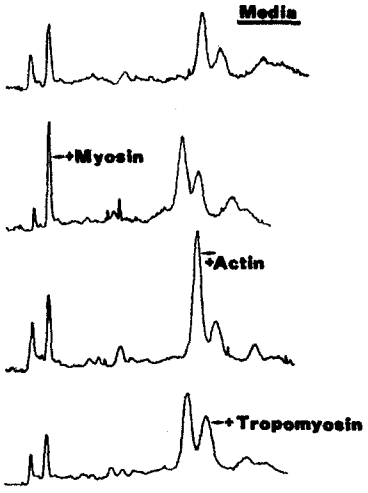


FIGURE 3

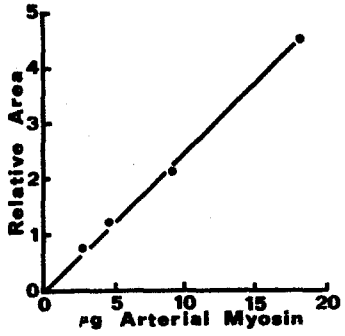


FIGURE 4

FIGURE 3. Tracings of densitometer scans of 24 μg of SDS-treated media homogenate with 5 μg of arterial myosin, actin, or tropomyosin added after electrophoresis on 7% acrylamide gels. Arrows indicate peaks enhanced by added protein. Origin of gels is at left. Vertical misalignment of peaks reflects small differences in migration rates on the separate gels.

FIGURE 4. Calibration showing relationship between areas under the peaks of densitometer tracings of 7% acrylamide gels corresponding to the myosin heavy chains as a function of micrograms of myosin applied to the gels. The line was drawn to pass through the origin.

molecular weight of 53,000 daltons (Fig. 5). If 8 M urea was merely included with SDS without preincubation, only 24–28% of this peak shifted. We conclude that about half the protein in the tropomyosin-containing peak (Fig. 3) represents tropomyosin. A roughly 19,000-dalton peptide also migrated more slowly after urea treatment (Fig. 5).

Although our purified protein samples showed the expected banding patterns after SDS treatment, myosin subunits can show aggregation under

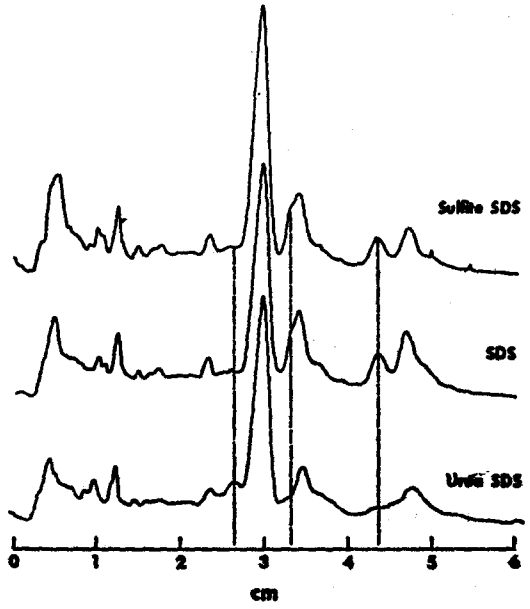


FIGURE 5. Densitometer tracings of SDS-treated arterial media homogenates. Top: 0.1 M sodium sulfite was included in the SDS mixture during homogenate treatment. Middle: standard procedure. Bottom: 8 M urea was included in the SDS mixture for homogenate treatment. Vertical dashed lines indicate portions of gel pattern altered by urea treatment at distances from the origin of 2.6, 3.3, and 4.4 cm corresponding to polypeptides of approximately 53,000, 36,000, and 19,000 daltons. These gels were more extensively polymerized than those shown in Fig. 3, giving greater resolution of peptides below 100,000 daltons.

some conditions on SDS gels (Paterson and Strohman, 1970). Consequently, it was possible that the most slowly migrating peak seen in Fig. 3 contained undissociated myosin chains and should be included in the myosin content estimates. However, sulfonation of the media preparation (Paterson and Strohman, 1970) to prevent this artifact did not alter any aspect of the SDS electrophoresis of the media homogenate (Fig. 5).

The estimates for maximum myosin, actin, and tropomyosin contents in the hog carotid media related to gram media wet weight, gram cell wet weight, or gram total protein, are given in Table II. The value for myosin

TABLE II
ESTIMATES OF PROTEIN CONTENT OF HOG CAROTID MEDIA BY DENSITOMETRY OF SDS-ACRYLAMIDE GELS

	Myosin	Actin	Tropomyosin
Media,* *mg/g*	9.7±1.4	30.2±4.0	8.4±2.0
Cell,‡ *mg/g*	16.2	50.3	14.0
Total protein,§ *mg/g*	85.2	265.4	74.0

* Values = mean ± 1 SEM for 5 preparations (the average results for 2–4 gels were used for each preparation).
‡ Values = mg/g media ÷ smooth muscle fraction of media (0.60).
§ Values = mg/g ÷ 0.1138 g protein/g media.

is comparable to those obtained on the basis of ATPase activity measurements (Table I).

Estimation of Myosin Content on the Basis of Extractable Protein

The amount of myosin which can be extracted and purified from arteries by our procedures was 0.97 ± 0.10 mg/g artery (N = 10 preparations). However, the media containing the smooth muscle cells represents only a fraction of the whole artery. This fraction was obtained by teasing away successive layers of adventitial connective tissue from five groups of 5–25 arteries/group so as to leave a tube consisting of the media and intimal layers corresponding to the tissue constituents of the strips used for physiological measurements. The ratio of media/artery wet weights was 0.362 ± 0.038. Therefore, the myosin yield can be computed as 2.7 mg/g media. However, even this value would underestimate the myosin content due to preparative losses. Such losses could be estimated fairly accurately after the initial fractionation through measurements of ATPase activity and examination of SDS gels of all protein fractions throughout the preparative procedure. The initial extraction of actomyosin from homogenized arteries was fairly quantitative as reported by Rüegg et al. (1965) although 25% more myosin can be obtained by exhaustive extraction at an ionic strength of 0.6. Low ionic strength precipitations of myosin as actomyosin, or by polyethylene glycol in the final step, were associated with a variable loss averaging 15 (±13, N = 6)%. However, the ultracentrifugal separation of actin from actomyosin was incomplete with a 57 ± 4% (N = 7) loss of myosin in the pellet. Corrections for losses allow an estimate of 9.9 mg myosin/g media, although this value is subject to considerable accumulated error.

DISCUSSION

The aim of this investigation was to compare the force-generating capacity of smooth and striated muscle in order to obtain new inferences about the contractile mechanism of smooth muscle. Absolute force development by the

media of the hog carotid artery under conditions of optimal stimulation, muscle length, and cell orientation is comparable to mammalian skeletal muscle. However, the extracellular space in carotid arteries is high. The value obtained (40%) was comparable with most other reports on carotid arteries: 25% in the dog (inulin space, Headings et al., 1960; sucrose space, Barr et al., 1962); 39% in the cow (thiosulfate space, Laszt, 1960); and 39% in the hog (electron microscope, Prosser et al., 1960); although problems of tissue shrinkage are difficult to assess (Burnstock, 1970). If the value obtained is approximately correct, the absolute force-generating capacity of the arterial smooth muscle cell appears significantly greater than that of striated muscle cells. There is a good correspondence between maximum contractile force in intact carotid arteries studied in vitro (Dobrin, 1973) and that generated by the teased media strips.

The high force measurements may indicate that smooth muscle has a contractile system which is specialized for force generation compared with skeletal muscle, although there are unknown factors which complicate this interpretation. For instance, the sum of the nonmyofibrillar space (sarcoplasmic reticular, nuclear, mitochondrial, etc.) in skeletal muscle may exceed that of smooth muscle, although this appears unlikely due to the large nuclear space in smooth muscle. The best comparison would require information about the ratios of thick and thin filaments, their density in the cell, and their lengths. Unfortunately, such information is unavailable because of the difficulties in fixing thick filaments for electron microscopy and the lack of a sufficiently ordered filament structure to yield solid data on filament lengths from longitudinal sections. However, information on the total content of myosin and actin in smooth muscle would provide an alternative basis for comparison.

Given the inherent methodological and experimental errors, the estimates of myosin content (milligrams per gram media) were remarkably uniform: 8.8 average for enzymatic methods, 9.7 by gel densitometry, and 9.9 by direct extraction with losses estimated by the first two methods. Because most methodological errors would produce overestimates, it seems likely that the myosin content is between 5 and 10 mg/g media, with 8 mg/g being a reasonable figure for further discussion.

The values for actin and tropomyosin contents are subject to greater uncertainty as they were obtained by SDS gel densitometry where the presence of other proteins in the peaks (in the presence and absence of TCA) would lead to overestimates. They can be estimated with the greatest accuracy as the ratio of the areas under the peaks containing actin or tropomyosin to that containing myosin in individual gels. These results calculated on the basis of 8 mg myosin/g media are given in Table III. The average ratio of actin to myosin obtained (3.18) compares with 3.85 and 2.27 for fresh and glycerol-extracted taenia coli, respectively, reported by Tregear and Squire

TABLE III
CONTRACTILE PROTEIN CONTENTS AND DISTRIBUTIONS IN STRIATED AND SMOOTH MUSCLE

Comparison	Rabbit skeletal			Arterial smooth		
	Myosin	Actin	Tropomyosin	Myosin	Actin	Tropomyosin
Tissue, *mg/g*	56*	20‡	4.5‡	8§	25‖	6‖
Cell,¶ *mg/g*	62	22	5 Σ = 89	13	42	11 Σ = 66
Total protein,** *mg/g*	336	120	27 Σ = 483	70	220	56 Σ = 346
Weight ratios	1.00	0.36	0.08	1.00	3.18	0.83
Mole ratios‡‡	1.00	3.52	0.54	1.00	31.10	5.57

* Average of values calculated from Marsten and Tregear, 1972 (55) and Helander, 1957 (57).
‡ Calculated from ratios to myosin summarized from literature by Ebashi and Nonomura, 1973.
§ Average of estimates from SDS gel, ATPase studies, and myosin extraction data.
‖ Calculated from ratios to myosin obtained from SDS gel data.
¶ Assuming extracellular space = 10% for striated and 40% for the smooth muscle.
** Based on 167 mg total protein per g tissue for skeletal (Helander, 1957) and 114 mg/g for carotid media.
‡‡ Based on an assumed molecular weight of 470,000 for myosin; 48,000 for actin, and 70,000 for tropomyosin.

(1973), and 2.5 obtained by S. Driska and D. J. Hartshorne (personal communication) in the chicken gizzard using similar methods. Some preparations were glycerol extracted to remove nonmyofibrillar proteins which could produce such errors, but the protein ratios were not altered.

Table III also gives comparative figures for rabbit skeletal muscle protein content based on values for myosin calculated from the literature (Marston and Tregear, 1972; Helander, 1957) and on ratios for other proteins summarized by Ebashi and Nonomura (1973). After correction for extracellular space, the sum of the myosin, actin, and tropomyosin in the smooth muscle cells is about three-fourths of the total in skeletal muscle cells. The comparison may be closer if corrections were made for nuclear space which is significant in smooth muscle cells. The striking difference between the two types of muscle is the protein distribution. There is a ninefold greater actin content relative to myosin in the smooth muscle based on the SDS gel estimates. The tropomyosin content is also very high in smooth muscle. However, the calculated molar ratio of actin monomers to tropomyosin is 5.6, a value which does not differ from the 6 actin monomers for each tropomyosin found in skeletal muscle (Lehman et al., 1972). High tropomyosin contents relative to the total myosin plus actin have been consistently reported in smooth muscle (Needham and Williams, 1963; Carsten, 1968; Hamoir and Laszt, 1962), but this primarily reflects the high relative actin content.

If the basic contractile system is similar in smooth and striated muscles, the potential number of cross bridges must be related to the myosin con-

tent. Our data indicate that for a given amount of myosin, the smooth muscle cell develops very nearly 10 times as much force. Clearly, the smooth muscle must either develop more force per cross bridge or the contractile proteins must be arranged in such a way that a much greater fraction of the total available cross bridges act in parallel. Important unknown information includes filament structure and lengths, the ratio of thick to thin filaments, and how the force developed by single cells is transmitted to the ends of the strip.

Our data give no direct way to estimate filament numbers and dimensions. However, thick and thin filament diameters are comparable in mammalian smooth and striated muscle (Somlyo et al., 1973; Burnstock, 1970; Kristensen et al., 1971; Bois, 1973). Although filament lengths vary greatly in different species, the length of thin filaments always appears proportional to that of thick filaments (Franzini-Armstrong, 1970). By assuming that the relative thin/thick filament lengths are comparable in mammalian smooth and skeletal muscle, the thin to thick filament ratio can be estimated knowing the actin/myosin weight ratios for the two types of muscle (Table III) and the filament number ratio for skeletal muscle (two thin/thick). The resulting prediction of 17.7 thin filaments for each thick filament in smooth muscle is very similar to the observed number of 15 (Somlyo et al., 1973) in the electron micrographs of the main pulmonary artery and portal-anterior mesenteric vein of the rabbit. A lower count of 12 thin/thick filaments was reported for the rat intestine (Bois, 1973), but counts from electron micrographs would tend to give lower ratios by not taking into account peripheral and distal regions of smooth muscle cells which have no thick filaments (Bois, 1973; Kristensen et al., 1971). The correspondence between thin/thick filament ratios predicted on the basis of protein content estimates and those observed in photomicrographs of well preserved tissues gives added confidence to the results of both approaches.

In conclusion, we have shown that the smooth muscle cell of the hog carotid artery can develop greater active tension per cell cross-sectional area than mammalian striated muscle cells. This occurs even though the total cellular myosin content, and therefore the potential number of cross bridges, is approximately one-seventh that of skeletal muscle (Table III). A better comparison of the force-generating capacity of smooth and striated muscles depends on detailed information about filament structure and organization.

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