Actin and Tropomyosin Variants in Smooth Muscles

DEPENDENCE ON TISSUE TYPE

Valerie Fatigati and Richard A. Murphy

From the Department of Physiology, University of Virginia School of Medicine, Charlottesville, Virginia 22908

(Received for publication, July 16, 1984)

Actin was found to be the major source of myofibrillar protein heterogeneity in smooth muscles. Three isolectric variants, α-smooth muscle (α-SM), β-nonmuscle (β-NM), and γ-actins (γ-SM and γ-NM) were measured in 15 different smooth muscles. α-SM and γ-actin contents displayed an inverse relationship in a given smooth muscle, some of which contained primarily α-SM actin while γ-actins dominated in others. α-SM actin and γ-actin distributions were tissue-specific, independent of species. A greater proportion of α-SM actin appears to be associated with tissues having a high degree of tonic activity. β-Nonmuscle actin was a significant, and relatively constant, component of all smooth muscle tissues. The high NM-actin content of these tissues may reflect the importance of proliferative, synthetic, or secretory activities in smooth muscle, because the α-SM actin disappeared in tissue culture with a time course paralleling the modulation of phenotype from a contractile to a proliferative cell. Two tropomyosin subunits were present in approximately equal amounts in all smooth muscle tissues studied. One tropomyosin subunit exhibited identical mobility on two-dimensional gel electrophoresis, while the other was characterized by some species-specific variation which was unrelated to actin variant distribution. No variants of the 20,000-dalton regulatory light chain of myosin were observed. These results suggest that SM-specific actin variants are associated with functional diversity among smooth muscles.

Myofibrillar protein variants have been observed in smooth and striated muscles. Myosin variants exhibit differences in specific ATPase activity in striated muscles which are proportional to the maximum velocity of shortening for zero load (Bárany, 1967; Weeds, 1978). Differences in the ratio of α/β tropomyosin subunits have also been observed between fast and slow striated muscles (Cummins and Perry, 1973, 1974; Johnson, 1974), although the biological significance of this is unknown. Skeletal and cardiac muscles exhibit no significant variability in the actin content or type.

There is little evidence for myosin or tropomyosin variants in smooth muscle (Murphy et al., 1983). However, four forms of actin have been detected in several smooth muscle tissues (Vandekerckhove and Weber, 1978). These include two variants specific to smooth muscle (α-SM and γ-SM actin) plus appreciable amounts of the two cytoplasmic actins found in virtually all eukaryotic cells (β-NM and γ-NM actin). The possible relationship of actin variants to smooth muscle function is not known.

The aim of this study was to determine whether the distribution of actin variants and/or the presence of myosin light chain or tropomyosin subunit variants is associated with differences in smooth muscle function in a series of smooth muscles, many of which have been the subject of extensive mechanical characterization.

MATERIALS AND METHODS

Preparations

The criteria for tissue selection were as follows: 1) ability to dissect smooth muscle layers relatively free of nonmuscle cells which would bias contractile protein measurements; 2) availability of mechanical data and/or estimates of total actin, tropomyosin, and myosin contents; and 3) variety, allowing species- and tissue-specific comparisons.

Tissue Preparation—Tissues were collected from animals at slaughter or when experiments with animals were terminated and stored in a swineoinic-buffered physiological salt solution at 4 °C until used (0–2) days. Complete viability of the tissues as assessed by measurements of mechanical or contractile properties can be maintained over this period (Dillon and Murphy, 1982). The composition (mM) of the physiological salt solution was NaCl, 140; KCl, 4.7; MgSO4, 1.2; CaCl2, 1.6; NaHPO4, 1.2; n-glucose, 5.6; EDTA, 0.02; and MOPS, 2.0 (pH 7.4 at 37 °C). The smooth muscle layers were carefully dissected, lightly blotted, and weighed. Tissue samples were frozen in an acetone/dry ice slush and homogenized in 1% SDS (10 mg of tissue/ml of SDS homogenization medium) according to the method described by Aksouy et al. (1983). Polypeptide resolution was optimal when the samples were immediately subjected to electrophoresis.

Isolated Smooth Muscle Cells—Smooth muscle layers from swine aorta were aseptically dissected from the tissues. The cells were enzymatically isolated from minced samples (Chamley-Campbell et al., 1979). Cells were plated at a density of 5 × 10⁴ cells/cm² for culture and grown in M-199 medium (Gibco) containing 10% fetal calf serum (Gibco), 100 units/ml of penicillin and 100 µg/ml of streptomycin (Gibco) (Chamley-Campbell et al., 1979). Cells were observed at different times from plating until confluence. Harvested cultured primary smooth muscle cell isolates were washed in phosphate-buffered saline and counted using a hemocytometer. The cell pellet was resuspended in SDS homogenization medium at 37 °C (2,000 to 20,000 cells/µl) and sonicated.

Resolution of Protein Variants

A modification of O'Farrell's (1975) two-dimensional electrophoresis method was used in which IEF was followed by electrophoresis on a polyacrylamide slab containing SDS. See Fatigati (1983) for additional details regarding optimization of these procedures.

* This work was supported by National Institutes of Health Grant 5 P01 HL19242. This study describes portions of a Ph. D. dissertation (University of Virginia, 1983) by V. F. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 The abbreviations used are: SM, smooth muscle; NM, nonmuscle; SDS, sodium dodecyl sulfate; MOPS, 4-morpholinepropanesulfonic acid; IEF, isoelectric focusing.
**Protein Variants in Smooth Muscles**

**Isoelectric Focusing**—The IEF gels (3 mm x 18 cm) were cast according to O'Farrell's method (1975) except that the ampholyte mixture was restricted to the 4–6.5 interval. The gels were allowed to polymerize for at least 2 h and were prefocused at 18 °C using a SDS homogenization medium overlay for 15 min at 300 V, 30 min at 650 V, and 30 min at 1000 V. Water at 18 °C was circulated through the cooling core during prefocusing and focusing. Samples were loaded onto the gel, focused and gels in varying amounts to optimize visualization of specific proteins as follows: 3–20 µl to estimate actin, desmin, and vimentin; 40 µl for tropomyosin; and 150–200 µl for the 20,500-dalton myosin light chain. The sample was overlaid with 20 µl of SDS homogenization medium, and fresh 0.02 M NaOH was used to fill the tubes. The samples were focused at 1000 V for 16 h. The gel tube was baked with a Bio-Rad Gel Tube Eliminator and the gel segment containing the protein(s) to be studied was either placed immediately on a SDS slab gel or sealed in a glass tube and rapidly frozen in an acetone/dry ice slush for analysis within two weeks.

**SDS Electrophoresis**—The polyacrylamide gels were separated in the second dimension according to electrophoretic mobility in SDS in 12% polyacrylamide slabs. A modification of O'Farrell's (1975) procedure for "nonequilibrated gels" (Driska et al., 1981) was followed except that electrophoresis with the "high-SDS-running buffer" was reduced from 20 to 10 min. The gels were stained overnight in 1 liter of 25% isopropanol, 10% acetic acid, and 0.04% Coomassie Brilliant Blue R (Fairbanks et al., 1971). Gels were first destained in 25% isopropanol and 10% acetic acid for 5 h and then transferred to 10% acetic acid for complete destaining. Gels were either photographed or dried after densitometry.

**Scanning Densitometry and Data Analysis**—The gels were scanned with a Quick Scan Jr densitometer which was custom modified with high resolution optics (Helena Laboratories) at a wavelength of 540 nm. A pinpoint light source was used. The protein spot must be scanned through the center in both electrophoretic dimensions to obtain the maximum optical density. Several scans were made to insure that the maximum optical density was obtained. Gels were excluded from data analysis if the scans were not symmetrical.

The densitometer peak heights were measured together with the peak widths at one-half peak height. The optical density value \( A \) was calculated by the following equation (Fatigati, 1983) \( A = 4/3 \times W_{gg} \times W_{sd} \), where \( H = \) maximum peak height, which should be equal for scans in the IEF and SDS directions. The larger value was used if slight variations occurred. \( W_{gg} \) and \( W_{sd} \) are the widths of the peaks at the half-maximum height for the scan in the IEF and SDS direction, respectively. The optical density values for each variant were converted into a per cent of the total protein optical density for all variants of a specific protein and the results were averaged (± S.E.).

Table 1 illustrates the reproducibility of data obtained for actin with this method. Comparison of the errors for estimates for individual tissues (A) with errors calculated from data obtained with different samples (B) indicates that the method is reasonably reproducible, but it is a major source of variability. Comparable results were obtained for tropomyosin subunit comparisons.

**Identification of Proteins after Electrophoresis**

The myofilbrillar proteins are the major polypeptides present in smooth muscle cells and are readily observed (Fig. 1). Three isolectric variants of actin can be separated. Desmin, vimentin, tropomyosin (two subunits), and the 20,000-dalton myosin light chain are also resolved with the appropriate loading.

The actin variants were tentatively identified by comparison with published results (Garrels and Gibson, 1976; Izant and Lazarides, 1977). The following studies were conducted to insure that the three spots were different actins and not charge modifications. Rabbit \( \alpha \)-skeletal muscle actin, prepared according to the method of Spudich and Watt (1971), appeared as one spot in the gel. Thus, the method did not lead to detectable charge modifications which would be confused with true isoelectric variants. The \( \alpha \)-skeletal muscle actin coelectrophoresed with the most acidic actin variant from smooth muscle tissue, thereby identifying it as \( \alpha \)-SM actin. The consistency in results (Table 1) also argues against significant charge modifications during tissue processing and electrophoresis. The \( \beta \)-NM and the unresolved \( \gamma \)-SM and \( \gamma \)-NM variants were confirmed by coelectrophoresis of various smooth muscle tissues and endothelial cells. Tropomyosin and the phosphorylated and nonphosphorylated forms of the 20,000-dalton myosin light chain were previously identified in this gel system (Driska et al., 1981).

**Statistics**

The t-test was used to determine if values were members of the same population (Snedecor, 1956). Mean values were considered significantly different at \( p \leq 0.05 \). Calculation of the actin variant content as mg/g of cell wet weight required multiplying the total actin content (either individual tissue values or average arterial or nonarterial values) by the relative actin variant composition (as per cent of total actin). The S.E. for actin variant contents was calculated from the following equation (Day and Underwood, 1967):

\[
S.E. = \sqrt{(\tau X S.E.)^2 + \gamma (S.E.)^2}
\]

where \( x = \) total actin estimate (mg) and \( y = \) per cent of total actin estimate for each variant.

**RESULTS**

**Distribution of Actin in Smooth Muscle Tissues**—For a given tissue type, irrespective of species (i.e. all arterial smooth muscles, all tracheal smooth muscles), the \( \alpha \)-SM actin was a fairly constant fraction of the total actin (Table II). Across tissue types, a continuum of \( \alpha \)-SM actin fractions was observed (Table II). Interestingly, there is a difference in \( \alpha \)-SM actin values between the lower esophageal sphincter and the adjacent, anatomically indistinguishable circular layer of the esophagus in cat and opossum. These tissues can only be

| TABLE I | Reproducibility of the two-dimensional gel electrophoretic method for quantifying proteins | Values are per cent total actin (± 1 S.E.) where n represents the number of measurements. |
|---------|-----------------------------------|-----------------------------------------------|
| A. Repeated measurements on one sample | B. Measurements on different samples |
| \( \alpha \)-SM | \( \beta \)-NM | \( \gamma \) | \( \alpha \)-SM | \( \beta \)-NM | \( \gamma \) |
| **Tissue** | | | | | |
| Swine carotid | 54.6 ± 0.7 | 16.9 ± 0.7 | 28.5 ± 1.1 | 7 | 58.2 ± 1.9 | 20.8 ± 1.9 | 21.0 ± 2.4 |
| Rabbit trachealis | 41.0 ± 1.7 | 27.6 ± 0.7 | 31.4 ± 2.4 | 5 | 39.7 ± 3.0 | 29.3 ± 0.9 | 31.0 ± 2.7 |
| **Cells** | | | | | |
| Swine aorta* | 61.9 ± 2.6 | 22.2 ± 2.3 | 15.9 ± 0.8 | 5 | 56.1 ± 2.3 | 25.1 ± 1.0 | 18.5 ± 1.3 |

* One day in primary culture.
distinguished on the basis of contractile activity.

The \( \gamma \cdot (\gamma\text{-SM and } \gamma\text{-NM}) \) actin content also exhibited a continuum of values across tissue types (Table II) and increased as the \( \alpha\text{-SM actin fractions fell. There was more variation in } \gamma \text{-actin content for a given tissue type than in the } \alpha\text{-SM fraction. This variability may arise from the combination of } \gamma\text{-NM and } \gamma\text{-SM actins which are not resolved. } \beta\text{-NM actin constituted a significant component in all tissues. The overall tissue average (18.6 ± 1.7%) appears to be too high to be totally ascribed to the actin content of nonmuscle cells in the tissues.}

**Actin Content of Smooth Muscle Cells**—The basis for the high \( \beta\text{-NM content in smooth muscle tissues (compared to striated muscle, where it is virtually undetectable) prompted two sets of experiments. Primary cell isolates prepared for standard tissue culture of smooth muscle should have a reduced fraction of nerves or perhaps other cell types which might contribute to a high NM-actin content. However, swine aortic primary isolates showed no differences from the dissected tissue preparations (Table III). This was also true for swine tracheal primary isolates subjected to further fractionation on a Percoll gradient (Table III). The aortic smooth muscle cells lost the \( \alpha\text{-SM actin variant in culture over the period associated with the proliferation and growth to confluence (Fig. 2). However, passaged cells did contain } \alpha\text{-SM actin (approximately 18%, } n = 1 \text{). These experiments suggest that the NM actins are a normal constituent of smooth muscle cells and that their presence can be reasonably estimated in tissues selected according to the criteria established for this study.}

The absolute content (mg/g of smooth muscle cell, wet weight) for the actin variants was estimated by multiplying the per cent composition of each actin variant by the previously estimated total cellular actin content (mg/g of smooth muscle cell, wet weight) for each tissue (Fig. 3). The error for each value used in this calculation was taken into account (see “Materials and Methods”). The variability reported for the total mg of actin/g of cell, wet weight, values can be large due to propagation of errors in content and in cell fraction estimates (Table II). Arteries have a significantly higher actin

### Table II

| Tissue (cell fraction ± S.E.) | \( n \) | Total actin (± S.E.) |
|-----------------------------|--------|---------------------|
|                            |        | \( \alpha\text{-SM} \) | \( \beta\text{-NM} \) | \( \gamma\text{-SM + } \gamma\text{-NM} \) | \( \alpha/\gamma \) Ratio |
| Aorta, swine (0.44 ± 0.03)* | 4      | 59.3 ± 1.8          | 22.4 ± 1.4          | 18.3 ± 1.3          | 3.2               |
| Carotid, swine (0.56 ± 0.02)* | 7      | 58.2 ± 1.9          | 20.8 ± 1.9          | 21.0 ± 2.4          | 2.8               |
| Carotid, dog (0.44 ± 0.05)  | 5      | 58.4 ± 1.8          | 13.6 ± 1.6          | 28.1 ± 2.8          | 2.1               |
| Renal vein, swine (0.40)*   | 3      | 46.2 ± 3.1          | 18.7 ± 1.7          | 35.1 ± 4.8          | 1.3               |
| Trachealis, swine (0.71 ± 0.04)* | 4 | 39.4 ± 3.2          | 14.0 ± 2.2          | 46.6 ± 2.7          | 0.8               |
| Trachealis, dog (0.60)*     | 5      | 37.3 ± 3.3          | 21.2 ± 1.8          | 41.5 ± 3.5          | 0.9               |
| Ureters, swine (0.60 ± 0.02)* | 5     | 39.7 ± 3.0          | 29.3 ± 0.9          | 31.0 ± 2.7          | 1.3               |
| Lower esophageal sphincter, cat | 4 | 30.6 ± 2.5          | 17.7 ± 1.7          | 51.8 ± 3.7          | 0.6               |
| Lower esophageal sphincter, opossum | 1 | 33.0               | 20.0               | 46.5               | 0.7               |
| Esophagus, swine (0.87 ± 0.02)* | 4 | 20.7 ± 3.7          | 18.1 ± 1.4          | 61.1 ± 2.8          | 0.3               |
| Esophagus, cat              | 2      | 15.4 ± 1.3          | 15.9 ± 0.1          | 68.8 ± 1.2          | 0.2               |
| Esophagus, opossum          | 1      | 13.1               | 11.2               | 75.7               | 0.2               |
| T. coli, guinea pig (0.64)*  | 3      | 16.7 ± 3.0          | 23.6 ± 2.6          | 59.7 ± 5.5          | 0.3               |
| Jejunum (circular), cat (0.87 ± 0.03) | 3 | 5.3 ± 1.4          | 10.3 ± 1.9          | 84.4 ± 3.2          | 0.1               |

* Cohen and Murphy (1978).

* Cohen and Murphy (1979).

* Stephens et al. (1969).

* Lowy and Mulvany (1973).

### Table III

| Tissue (cell fraction ± S.E.) | \( n \) | Total actin (± S.E.) |
|-----------------------------|--------|---------------------|
|                            |        | \( \alpha\text{-SM} \) | \( \beta\text{-NM} \) | \( \gamma\) |
| Aorta                      |        | 59.3 ± 1.8          | 22.4 ± 1.4          | 18.3 ± 1.3          |
| Tissue                     | 4      | 56.1 ± 2.3          | 25.1 ± 1.0          | 18.5 ± 1.3          |
| Trachealis                 | 4      | 39.4 ± 3.2          | 14.0 ± 2.2          | 46.6 ± 2.7          |
| Cells*                     | 2      | 36.7 ± 4.0          | 22.9 ± 1.8          | 40.4 ± 2.2          |

* Primary isolates.

* Primary isolates after purification on Percoll gradient.
content than have other smooth muscle cells (Cohen and Murphy, 1978, 1979). However, no significance can be attributed to differences between total tissue actin contents in other tissues.

The "extra" actin in arterial smooth muscle compared with all other smooth muscle types examined (Cohen and Murphy, 1978, 1979; Fatigati et al., 1982) was primarily due to a high α-SM actin content (Fig. 3).

Tropomyosin—The two tropomyosin subunits differed in their isoelectric points as well as in their molecular weights and were labeled + (basic) and − (acidic) accordingly (Fig. 4). The subunits were present in equal amounts in the rabbit trachealis and cat jejunum (Table IV). The results for other tissues showed slightly greater amounts of the positive subunit. This imbalance may reflect relative staining differences, the presence of another protein co-migrating with the positive subunit, excess synthesis of the positive subunit, differential proteolysis during sample processing, or the presence of tropomyosin molecules composed of two positive chains. However, the molar ratio of actin to tropomyosin for all tissues was $5.94 \pm 0.74$. This is comparable to the value determined for thin filaments from striated muscles (Cohen and Murphy, 1978, 1979) and suggests that the overall quantification of actin and tropomyosin is reasonable.

The more positive tropomyosin subunit was common to all tissues studied (Fig. 4), as judged by coelectrophoresis. Swine carotid, trachealis, aorta, and esophageal smooth muscle, together with the cat jejunum, rabbit trachealis, and guinea pig taenia coli all appeared to have the same two subunits. Dog carotid media and trachealis had identical subunits. The negative subunit of dog tissues had a slightly higher molecular weight than the negative subunit of the other tissues, and it may represent a species-specific variant. No tissue-specific variation or correlation with the distribution of actin variants was apparent in the tropomyosin studies. Comparison of tropomyosin subunits from swine carotid media with dog hind

Tropomyosin Subunit Variants in Smooth Muscles

![Fig. 2. Change in the actin composition of swine aorta smooth muscle cells during culture. The fractional content of the actin variants for swine aortic tissue (T) was compared with enzymatically dispersed primary isolates before (0 days) and during culture.](image)

![Fig. 3. Cellular actin variant content. The top of each bar indicates the total actin content (Cohen and Murphy, 1978, 1979; Fatigati et al., 1982). The length of each segment within a bar indicates the amount of each actin variant (± S.E.). The smooth muscle cell fractions used for these estimates are listed in Table II.](image)

![Fig. 4. Tropomyosin subunit variants in smooth muscles. Two tropomyosin subunits (+ and −) were detected in all tissues studied. Coelectrophoresis distinguished the canine acidic variant from other species.](image)
skeletal muscle tropomyosins had no subunits in common. exhibit any tissue indicated that the 20,000-dalton myosin light chain did not the heavy chains were poorly resolved on the gel system and tissues were dissected and homogenized in solutions which did not cause contraction. The 17,000-dalton light chain and phos- carotid media, cat jejuneum, guinea pig taenia coli, swine carotid media, trachealis, and esophagus. The phos- phorylated form of the 20,000-dalton myosin light chain was only seen occasionally. This was not surprising since the tissues were dissected and homogenized in solutions which did not cause contraction. The 17,000-dalton light chain and the heavy chains were poorly resolved on the gel system and were not analyzed.

**DISCUSSION**

The molecular basis for functional differences among vertebrate striated muscles lies in the expression of different isoenzymatic forms of myosin. Smooth muscles exhibit even greater diversity in activity patterns. Nevertheless, there is no compelling evidence for the existence of different isoenzymes of myosin among smooth muscles, or that they contribute to functional specializations (Murphy et al., 1983). This study has demonstrated that smooth muscles exhibit a unique type of molecular diversity in the contractile proteins, i.e. tissue-specific patterns in the contents of isoactins. The possible functional significance of these distributions is considered below.

**Significance of Nonmuscle Actins in Smooth Muscle Tissues**—The relative \( \beta \)-NM actin content ranged from 10.3–33.3% of the total actin. This range may reflect differences among smooth muscle cells and/or the actin content of varying amounts of nonmuscle cell types in the tissues. The particularly high fractional amounts of \( \beta \)-NM actin observed in some tissues may reflect nonmuscle cells. For example, nonpregnant myometrium (35.3% \( \beta \)-NM actin) is perhaps the tissue most likely to have the highest fraction of nonmuscle cells among those studied. Furthermore, the estimated \( \beta \)-NM actin content decreased with the size of the three smooth tissues, where greater size facilitated dissection of the more purely muscular portions of the tissue. At the other extreme, the cat intestinal circular muscle had a very high smooth muscle cell content and a comparatively low \( \beta \)-NM fraction (Table II). These observations are consistent with the hypothesis that nonmuscle cells contribute significantly to the \( \beta \)-NM actin content of many tissues. The \( \beta \)-NM actin content is not tissue-specific, as shown by the variation observed among smooth muscles in a given type (10.3–23.6% for gastrointestinal smooth muscle, 13.6–22.4% for arterial smooth muscle, and 14.0–29.3% for trachealis smooth muscle).

While it seems probable that nonmuscle elements may contribute to estimates of \( \beta \)-NM actin, the results also provide fairly strong evidence that \( \beta \)-NM actin is a normal constituent of smooth muscle cells because (a) many of the tissues appear to contain almost entirely smooth muscle cells on a volume basis, and (b) isolated smooth muscle cells (Table III) have appreciable amounts of \( \beta \)-NM actin.

In contrast to smooth muscle, skeletal muscle has virtually no nonmuscle actins (less than 1%; Pardo et al., 1983). It is possible that nonmuscle actins reflect aspects of smooth muscle function. Smooth muscle is not a dedicated contractile cell and has significant proliferative, synthetic, and secretory functions (Wissler, 1968). The nonmuscle actin may therefore be associated with these noncontractile functions. This interpretation was supported by the cell culture experiment (Fig. 2). Vascular smooth muscle cells in primary culture lose their thick filaments (and apparently their ability to contract normally) and develop an increased content of organelles associated with protein synthesis and secretion during the proliferative phase (Chamley-Campbell et al., 1979). The time course of change in actin distribution during culture of swine aortic smooth muscle cells (Fig. 2) appears to reflect this change in cell phenotype and function (Franke et al., 1980). The most reasonable interpretation of our results is that some, and perhaps 25%, of the actin in smooth muscle may reflect the expression of genes involved with basic cellular activities not associated with the contractile apparatus. This interpretation is consistent with observations suggesting that appreciable amounts of actin in smooth muscle are not polymerized (Murphy and Megerman, 1977; Seidel et al., 1981) and presumably complexed with various actin-binding proteins. The analysis omits the contribution of \( \gamma \)-NM actin which was not resolved from \( \gamma \)-SM actin by isoelectric focusing. However, the \( \beta \)-variant is likely to be the predominant nonmuscle actin form (see below). Cell culture studies of smooth muscle have been seriously hampered by the absence of readily detectable markers for the state of cytodifferentiation. The isoactin distribution estimated by two-dimensional gel electrophoresis may be of value as such a marker. 

| Tissue                | n | Total tropomyosin (± S.E.)   |
|-----------------------|---|-----------------------------|
|                      |   | +Subunit        | -Subunit       |
| Swine carotid        | 7 | 53.1 ± 1.4  | 46.9 ± 1.4*   |
| Dog carotid          | 7 | 59.8 ± 2.7  | 40.2 ± 2.7*   |
| Dog trachealis       | 8 | 56.5 ± 2.9  | 45.3 ± 2.9*   |
| Rabbit trachealis    | 3 | 48.2 ± 0.8  | 51.8 ± 0.8    |
| Guinea pig T. coli   | 7 | 54.2 ± 1.4  | 45.8 ± 1.4*   |
| Cat intestine        | 4 | 49.2 ± 1.8  | 50.8 ± 1.8    |

* Significantly different at \( p < 0.025 \).
It is evident that the “extra” actin in arteries is due to a high content of α-SM actin. It is this difference in content which produces high α/γ actin ratios in arteries. The experimental data (Table II) do not provide any clear basis for defining any further general categories based on isoactin distributions. The following analysis considers whether any pattern in functional behavior parallels the data.

Actin variant distributions or total actin contents do not show any obvious relationships with published estimates of contractile function such as maximum active stress or shortening velocity. However, most of the velocity and stress values in the literature probably do not reflect the true contractile system capacity. Stress measurements are typically made at submaximal activation, and shortening velocity is affected by the level of myosin phosphorylation (Murphy et al., 1983). These factors may obscure any correlations with parameters reflecting aspects of the cross-bridge interaction such as maximal stress generating capacities or maximal shortening velocities.

There does appear to be a general relationship between activity patterns and isoactin distributions, however. Muscles that are normally relaxed (i.e. esophageal body muscles and intestine) contain primarily γ-actin (presumably γ-SM). In contrast, muscles which are normally contracted (arteries and lower esophageal sphincter) contain greater amounts of α-SM actin. The terms “normally relaxed” and “normally contracted” were used rather than phasic and tonic, because the latter classification is usually based on the in vitro behavior of tissues. The normal physiological contractile state is uncertain for some of the muscles tested. For example, the trachealis may normally exhibit tone or, alternatively, its primary activity may involve phasic contractions as part of reflexes triggered by airway irritants. Comparison of the lower esophageal sphincter (normally contracted) and the adjacent, anatomically indistinguishable circular layer of the esophagus in cat and opossum provided a test of the general relationship. As predicted, the normally contracted lower esophageal sphincter muscle and corresponding normally relaxed esophageal body muscle contained greater amounts of α-SM actin. It is this difference in content which produces high α/γ actin ratios (Table II). The sphincter ratios are somewhat lower than those of the tonic arteries. However, sphincter tissues are identified only by the development of tone when placed in a tissue bath, and our tissue samples undoubtedly contained some esophageal body muscle, which would lower the ratio.

The results are consistent with the hypothesis that the tissue-specific actin distribution is correlated with the amount or pattern of the contractile activity of the smooth muscle tissue. In skeletal muscle there is fairly strong evidence that myosin gene expression is regulated by the amount of contractile activity, with the fast myosin isoenzyme being expressed in cells which contract infrequently (Weeds, 1978). There is also considerable evidence suggesting that the expression of myosin isoenzymes in the heart depends on loading (Litten et al., 1982). It may be that stress or some cellular factor associated with contractile activity determines actin gene expression in smooth muscles. If this is true, it is a new aspect of differentiation and diversity in muscle. An important unanswered question is whether specific actin variants are associated with differences in the cross-bridge cycle and contractile function, and the nature of any such differences.

Acknowledgments—Special thanks are due to Shen-Valley Packing Company of Timberville, VA for donating the swine tissues, Dr. G. K. Owens for assistance with the cell culture experiments, Dr. D. M. Cohen for contractile protein content measurements, Dr. N. W. Weisbrod, for cat and opossum esophageal body and sphincter tissues, Betty Haigh for manuscript preparation, and Mildred Smythies for technical assistance.

REFERENCES

Aksoy, M. O., Mras, S., Kamm, K., and Murphy, R. A. (1983) Am. J. Physiol. 245, C255–C270
Bakr, A. N. (1967) J. Gen. Physiol. 50, 197–218
Chamley-Campbell, J., Campbell, G. R., and Ross, R. (1979) Physiol. Rev. 59, 1–61
Cohen, D. M., and Murphy, R. A. (1978) J. Gen. Physiol. 72, 369–380
Cohen, D. M., and Murphy, R. A. (1979) Circ. Res. 45, 661–665
Cummins, P., and Perry, S. V. (1973) Biochem. J. 133, 785–777
Cummins, P., and Perry, S. V. (1974) Biochem. J. 141, 43–49
Day, R. A., and Underwood, A. L. (1967) Quantitative Analysis, 2nd ed., p. 64, Prentice Hall, Englewood Cliffs, NJ
Dillon, P. F., and Murphy, R. A. (1982) Am. J. Physiol. 242, C102–C108
Driska, S. P., Aksoy, M. O., and Murphy, R. A. (1981) Am. J. Physiol. 240, C222–C233
Fairbanks, G., Steck, T. L., and Wallach, D. F. H. (1971) Biochemistry 10, 2806–2817
Fatigati, V. (1983) Contractile and Structural Protein Variants in Smooth Muscle, Ph. D. Dissertation, University of Virginia, University Microfilms, Ann Arbor, MI
Fatigati, V., Cohen, D. M., and Murphy, R. A. (1982) Fed. Proc. 41, 978 (abstr.)
Franke, W. W., Schmid, E., Vandenckhove, J., and Weber, K. (1980) J. Cell Biol. 87, 584–600
Gabbiani, G., Schmid, E., Winter, S., Chapronnier, C., deChastotnay, C., Vandenckhove, K., Weber, K., and Franke, W. W. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 293–302
Garrels, J. L., and Gibson, W. (1976) Cell 9, 793–805
Gerthoffer, W. T., and Murphy, R. A. (1983) Am. J. Physiol. 244, C129–C187
Izant, J. G., and Lazarides, E. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 1450–1454
Litten, L. S. (1974) Biochem. Biophys. Acta 371, 219–225
Litten, R. Z., III, Martin, B. J., Low, R. B., and Alpert, N. R. (1982) Circ. Res. 50, 856–864
Lowy, J., and Mulvany, M. J. (1973) Acta Physiol. Scand. 88, 123–136
Murphy, R. A., Aksoy, M. O., Cohen, D. M., Fatigati, V., and Gerthoffer, W. T. (1983) in Vascular Neuroeffector Mechanisms: 4th International Symposium (Bevan, J. A., Maxwell, R. A., Shiba, S., Fujinawa, M., Mohri, K., and Toda, N., eds) pp. 37–45, Raven Press, New York
Murphy, R. A., and Megerman, J. (1977) in The Biochemistry of Smooth Muscle, (Stephens, N. L., ed) pp. 473–498, University Park Press, Baltimore
O’Farrell, P. H. (1975) J. Biol. Chem. 250, 4007–4021
Pardo, J. V., Pittenger, M. F., and Craig, S. W. (1983) Cell 32, 1093–1103
Seidel, C. L., Snabes, M., and McLeod, J. (1981) Physiologist 24, 120
Snedecor, G. W. (1956) Statistical Methods, 5th Ed., pp. 46, Iowa State University Press, Ames, IA
Spudich, J. A., and Watt, S. (1971) J. Biol. Chem. 246, 4895–4851
Stephens, N. L., Kroeger, E., and Mehta, J. A. (1969) J. Appl. Physiol. 101, 685–692
Underwood, E. E. (1970) Quantitative Stereology, p. 18, Addison-Wesley Publishing Co., Inc., Reading, MA
Vandenckhove, J., and Weber, K. (1978) J. Mol. Biol. 126, 783–802
Vandenckhove, J., and Weber, K. (1979) Differentiation 14, 123–133
Vandenckhove, J., and Weber, K. (1981) Eur. J. Biochem. 113, 595–605
Weeds, A. (1978) Nature (Lond.) 274, 417–418
Wissler, R. W. (1963) J. Atheroscler. Res. 8, 201–213

N. L. Stephens, personal communication.