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ORGANIZATION OF CONTRACTILE UNITS IN SMOOTH MUSCLE BASED ON STEREOLOGICAL ANALYSIS OF DENSE BODY DISTRIBUTION

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ABSTRACT

It is generally assumed that smooth muscle (SM) cells contract by a sliding filament mechanism based on the structure of a contractile unit (cu) comprised of thick myosin filaments (mfs) and thin actin filaments (afs), which are anchored to cytoplasmic dense bodies (dbs) or membrane dense plaques (dps). Since the structure of the contractile apparatus is not readily apparent in SM, as it is in striated muscle, stereological estimates of structural parameters have been developed based on specific assumptions. The feasibility of measurements was tested on electron micrographs (EMs) of SM cells from sequential rabbit ear arteries. It is tentatively concluded that the number and length of cus aligned across the cell can be determined by measurements of relative areas of dps and dbs, and the orientation of dbs and filaments.

INTRODUCTION

Physiologically or functionally, a muscle contractile unit is the effector of chemomechanical transduction. This is accomplished by the interaction of afs and mfs such that they and other cellular components move relative to each other. This process has been well defined in striated muscle and is called the "sliding filament" mechanism. In striated muscle the afs insert at both ends of the cu into Z-discs. The mfs are usually situated between and interact with 2 afs. Thus, the relative amount of overlap of filaments (in the A band) changes with the amount of contraction.

Smooth muscles (i.e. without striation) have similar filaments and apparently exhibit a sliding filament form of contraction. However, a few aspects of SM force generation need to be more clearly defined as outlined by Murphy (1979).

Data from several types of SM are required on (a) myosin aggregate structures in vivo, together with thin- and thick-filament lengths; (b) the polarity of adjacent thin filaments on both sides of their anchor structures; (c) orientation of filaments with reference to the longitudinal axis of the cell and the resultant force vector; and (d) the number and relationships of thick and thin filaments interacting with each other in transverse register and forming a repeating contractile unit. The above list of required data leaves out reference to tissue, extracellular, and intercellular factors associated with force generation which are beyond the scope of this presentation.

A stereological approach for skeletal muscle, although complex, has been developed for describing intracellular structure (Eisenberg et al., 1974). The main aim of the present study is to indicate which stereological parameters can be accurately measured in order to provide data for the (c) and (d) in the above list. Several investigators have used or suggested three dimensional reconstruction and viewing as an approach for obtaining the necessary information (Bond and Somlyo, 1982; Fay et al. 1983; Tsukita et al., 1983). One purpose of this study is to see which parameters measured in two dimensional thin sections can be used to elucidate the structure of the contractile apparatus in three dimensions. For clarity the formulation is highly structured with evidence for each point included with the analysis. The feasibility of appropriate measurements is documented by a limited number of sample measurements and evaluated using some of the criteria outlined by Bolender et al. (1982).

ANALYSIS

Hypotheses 1. The number of contractile units across the cell N(cu) is a function of the area of db A(db) to area of dp A(dp) such that $N(cu) = 2\ A(db)/A(dp) + 1$. 2. The length of each cu l(cu) is related to the angle α with respect to the long axis of the cell and the diameter or width of the cell, W such that $l(cu) = W/N(cu)\sin\alpha$. 3. The number of dbs within the cell is $N_V(db) = 0.569\ N_A(db)^2/N_L(db)$.

Assumption 1.--actin filaments attach to dps linked to the membrane and dbs which are connected to the cytoskeleton by intermediate filaments (ifs) (Tsukita et al., 1983).

Assumption 2.--the polarity of afs is such that subfragment S-1 of mfs attaches with arrowheads pointing away from dp and in opposite directions from db. This implies that mfs can interact with afs as for striated muscle (sliding filament mechanism). Bond and Somlyo (1982) and Tsukita et al., (1983)

have demonstrated this S-1 decoration in vertebrate SM. The feasibility of the proposed cu is thus supported.

Assumption 3.--the number of attachments per unit area of db is two times greater for db (cytoplasmic) than it is for dp (membrane). It appears there is no direct support for this assumption, but it seems reasonable because of bidirectional afs from dbs and unidirectional afs from dps.

Assumption 4.--the number of cus across the cell N(cu) is proportional to (attachments into db)/(attachments into dp). Intuitively it is obvious that for each cu which ends in the cytoplasm there are attachments of actin into a db and for each ending at the membrane attachments occurs in the dp. Although possible oblique register and length of SM are considerations (Bagby and Kreyling, 1983) quantitation based on attachments avoids these two factors.

Conclusion 1. From assumptions 1-4 it follows that $N(cu) = 2\ A(db)/A(dp) + 1$. It remains to determine experimentally the areas for attachment of actin filaments but it is the prevailing view that the filaments are generally aligned along the cell (Gabella, 1979; Fay et al., 1983) so the transverse section of the cell would be adequate for measuring areas. Any slight parallax with filament angle can be shown by simple geometry to have no effect on average.

Corollary 1.1--if there are dps but no dbs, this implies that the contractile units extend completely across the cell, i.e. $N(cu) = 0 + 1$.

Assumption 5.--the contractile units are lined up in series such that they traverse the cell at an angle α with respect to the longitudinal axis of the cell. Fluorescence microscopy of dbs in single cells suggests an oblique angle of cus across the cell (Fay et al., 1983) while EM views of afs often indicate a more longitudinal alignment of afs (Gabella, 1979). The solid angle α could be approximated by the apparent angle α' in longitudinal sections by $\tan^2\alpha = 2 \tan^2\alpha'$.

Conclusion 2. For a cell with a diameter or effective width W and filament at a solid angle α the total cell intercept is $W/\sin\alpha$ and, thus in general, the average length of a contractile unit is $\bar{l}(cu) = W/N(cu)\sin\alpha$. It may be necessary to replace the term W by a more complex stereological relationship when and if it is determined how many cus terminate in the ends of the SM or do not cross the cell. For small solid angles $\alpha = 1.41\ [\alpha']$.

Assumption 6.--the cytoplasmic dbs generally have the shape of oblate spheroids for which the number per test area N_A and number per test line N_L can be counted. Recent observations of SM exposed to different treatments give the

impression that dbs are tapered cylinders or ellipsoids (Bond and Somlyo, 1982; Fay et al., 1983; Tsukita et al., 1983).

Conclusion 3. For the oblate spheroid shape described the volume density of contractile units is given by $N_V = 0.569 N_A^2/N_L$ as sampled from random sections for oblate spheroids with an axial ratio of b/a = 1/2 (Underwood, 1970).

METHODS AND MATERIALS

The SM structure of three different branching orders of arteries in ears from adult male New Zealand White rabbits was examined to test the feasibility of measurements for the above model analysis. The central ear artery (CEA), its main side branch (MSB), and a terminal branch (TB) were fixed, sectioned and stained for EM. The procedures and solutions required for isovolumetric preservation of SM as applied to these tissues are outlined by Walmsley et al.(1983).

Basic sampling procedures were used to determine if the hypothetical area measurements could be accomplished and the efficiency of these compared to measurements of other relavent stereological parameters. This evaluation was facilitated by employing a modified program of the PCS-I package on a Tektronix computer (Bolender et al., 1982). Five micrographs were chosen randomly from longitudinal sections of one artery from each classification. Because of the circumferential nature of SM alignment, these arterial sections provided approximately transverse sections of SM cells. A Weibel grid was superimposed on EM prints (8652X to 17304X) covering several cell profiles and resulting in a maximum of two point intersections on any one db or dp. Point counts gave $A_A = P_P$ for db, dp, nucleus and non-db cytoplasm. Line intersection counts were obtained for for db, dp, nucleus, dense plasma membrane, and non-dense plasma membrane.

Orientation of the contractile apparatus was observed in transverse sections of MSB arteries dilated at 100 mmHg. The resultant longitudinal cell sections showed profiles of afs combined with elongated dbs and dps. A total of 100 determinations were made of α' from 11 SM cells.

An attempt was made to determine the shape and axial ratio of dbs and dps in transverse and longitudinal sections.

RESULTS

As indicated in Table 1 the arteries have a 4 fold range in dilated internal diameter but the range of SM diameters is quite small (data from Walmsley et al., 1983). The total cell point counts indicated were from only five micrographs for each artery and resulted in the area measurements. The area standard error (SE) values are close to or below the 10% of the mean which is considered an acceptable level of sampling. In contrast, the SE curves for intersection counts which could have been useful for estimating the geometry of cus showed little indication of plateauing as analysed using PCS-I (data not shown). Furthermore, as indicated in the Table, the values of A(db) and to some extent A(dp) showed a remarkable

similarity from artery to artery considering the limited nature of the sampling. From Hypothesis 1, the predicted values for N(cu) tend to be greater for the larger SM cells.

TABLE 1

	CEA	MSB	TB
Internal diameter (μm ± SD, n=4)	960±114	456±47	251±10
SM cell diameter, W (μm, derived)	5.2	4.4	4.2
Total SM cell P_T (point counts)	4204	3592	3865
A(db) (% ± SE, n=5)	8.5±0.64	9.1±0.57	8.4±0.33
A(dp) (% ± SE, n=5)	4.4±0.31	5.4±0.57	6.5±0.53
N(cu) (μm) 2A(db)/A(dp)+1	4.87	4.36	3.59
α (° ± SE, n=100)		5.8±0.42	
$\underline{1}$(cu) (μm) W/N(cu)sinα	(10.6)	9.99	(11.6)

Measurements of α' for MSB cus resulted in a average orientation of -0.77° ± 5.03 (SD) with respect to the cellular axis or an average absolute angle of 4.11°± 2.98 (SD) or the solid angle approximation shown in Table 1. From Hypothesis 2, the length of the cu between dense areas in the MSB cells would be 10 μm. The values indicated in brackets for the other arterial classes are $\underline{1}$(cu) predicted from the MSB α.

Hypothesis 3 appears to be unworkable as tissue examined for this study had dbs and dps with irregular size and shape.

DISCUSSION

Arterial tissue prepared for EM under optimum conditions provides clear and distinct dbs and dps. Yet, since the shape of dense regions is variable, more general methods for estimating N_V than those stated in Hypothesis 3 may be required as discussed in other papers in this issue of Acta Stereologica. However, the ratio of A(db) and A(dp) can be measured from transverse sections at appropriate magnifications using point counting. The SE is less than 10% of the mean when using as few as 5 micrographs per artery. This area data has been inserted in the equation for Hypothesis 1 to infer that for the ear arteries sampled there are 3 to 5 cus across a cell. Yet, a review of cu models indicates several alternative arrangements (Bagby, 1983) and the use of dense area measurements would have a different meaning for different models. Mf, af or db alignment may be used to infer the cu angle α. These orientation data have been used with

Hypothesis 2 to approximate a cu length of 10μm for stretched MSB SM. The use of the cell diameter for W may not be appropriate as Gabella (1979) suggests that a higher proportion of the cell membrane is occupied by dp at the tapered ends and that at the level of the nucleus, the membrane has 30 to 50% dps. Regardless, the predicted value of $\underline{l}$(cu) is greater than the 2μm skeletal sarcomere length at optimum force and less than the 11μm estimate for the maximum length of afs (Murphy, 1979; Bagby, 1983) Because of the small α it may be useful to examine the change in orientation as a function of the cell length, L, normalized to the length for optimum contraction, L_o. Based on previous work (Walmsley et al. 1983) the dilated MSB used for the present study has SM at or slightly above L_o. It is clear that a wealth of information about the SM contractile apparatus can be obtained from fundamental stereological parameters in addition to the more prevalent reconstruction and whole cell visualizations.

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REFERENCES

Bagby RM. Organization of contractile/cytoskeletal elements. In: Stephens NL, ed. Biochemistry of smooth muscle. Vol. 1. Boca Raton, Florida: CRC Press, 1983:1-84

Bagby RM, Kreyling MC. Evidence for an obliquely organized myofibril in vertebrate smooth muscles. Fed Proced 1983; 42:736.

Bolender RP, Pederson EA, Larsen MP. PCS-I -- A point counting stereology program for cell biology. Computer Programs in Biomedicine 1982; 15:175-186.

Bond M, Somlyo AV. Dense bodies and actin polarity in vertebrate smooth muscle. J Cell Biol 1982; 95:403-413.

Eisenberg BR, Kuda AM, Peter JB. Stereological analysis of mammalian skeletal muscle. J Cell Biol 1974; 60:732-754.

Fay FS, Fujiwara K, Rees DD, Fogarty KE. Distribution of α-actinin in single isolated smooth muscle cells. J Cell Biol 1983; 96:783-795.

Gabella G. Smooth muscle cell junctions and structural aspects of contraction. Brit Med Bul 1979; 35:213-218.

Murphy RA. Filament organization and contractile function in vertebrate smooth muscle. Ann Rev Physiol 1979; 41:737-748.

Tsukita S, Tsukita S, Ishikawa H. Association of actin and 10 nm filaments with the dense body in smooth muscle cells of the chicken gizzard. Cell Tissue Res 1983; 229:233-242.

Underwood EE. Quantitative stereology, Addison-Wesley, Reading, Mass. 1970:90-91.

Walmsley JG, Owen MP, Bevan JA. Medial morphometry and mechanics of sequential rabbit ear arteries and myograph ring segments. Amer J Physiol 1983; 245:H840-H848.