Conduction in smooth muscle: comparative structural properties

C. L. PROSSER, GEOFFREY BURNSTOCK AND JOSEPH KAHN

Physiology Department, University of Illinois, Urbana, Illinois

Recent evidence strongly indicates that intercellular transmission in vertebrate smooth muscle is by some electrical means. Three models have been proposed: ephaptic transmission between overlapping cells (1), a morphological syncytium (2) and low resistance contacts between adjacent cells (3). Earlier theories postulated functional continuity with or without cytoplasmic continuity (4). The present papers consider these models by comparing the structural and electrical properties of visceral muscles which differ in velocity of conduction and in excitability.

Light microscopy was used for studying the arrangement of bundles, the number of fibers within bundles and fiber dimensions. Electron microscopy was used for studying the arrangement between cells, structure of cell membranes and of intracellular organelles and for estimating extracellular space.

METHODS

The following visceral muscles in which conduction occurs by nonnervous means were used: pig esophagus (muscularis mucosae), guinea-pig taenia coli, cat circular and longitudinal intestinal muscle, rat and guinea-pig ureters, dog retractor penis, rabbit bladder and guinea-pig vas deferens. Multitunit smooth muscles in which conduction is by nerves were: cat nictitating membrane, pig external carotid artery, renal vein and pulmonary artery and vein.

For light microscopy, muscles were fixed in Bouin's solution and stained with haematoxylin-eosin or were fixed in Zenker's solution or in formalin and stained with Mallory's triple stain. Fresh muscle was macerated in nitric acid-glycerine.

For electron microscopy, small strips of fresh muscle were relaxed in oxygenated Krebs' solution, then extended to twice this length and fixed. Fresh tissues and glycerinated bundles of fibers were fixed in Palade's solution, 1.2% osmium tetroxide in acetate-Veronal buffer, pH 7.5. They were dehydrated and embedded by the procedure described by Lund et al. (5). The material was sectioned on a Porter-Blum microtome and viewed with an RCA model EMU2 or EMU3 electron microscope. Representative sections are shown in the accompanying photographs. Fixation of blood vessels proved very difficult as compared with visceral muscles.

RESULTS

Observations by light microscopy. The fibers of some visceral muscles are arranged in bundles of irregular cross-section separated by thick bands of connective tissue (fig. 1 and table 1). The longitudinal muscle of the cat intestine has no bundles (fig. 1F) but the fibers of the circular layer are arranged in wide sheets which extend through the 500-μ layer and are slightly wider at the outer edge of the circular layer than at their base (fig. 1E). These sheets of the circular layer ranged from 112 to 406 μ (average 223 μ) wide. Connective tissue

545
strands penetrate the guinea-pig taenia from the side of its attachment to the intestine and divide it into bundles averaging 70 μ (range 50–90 μ) in width (fig. 1C). The dog retractor penis has bundles which are circular in shape and within which sub-bundles can be distinguished (fig. 1B). The average diameter of the large bundles in the dog retractor was 328 μ and the smaller sub-bundles 112 μ. In the esophagus muscularis mucosae the bundles are oval and variable in size, averaging 125 by 245 μ in cross-section; sub-bundles are less distinct than in the dog retractor penis (fig. 1D). In both these muscles and in the taenia there is branching and fusion between the sub-bundles at close intervals (0.5–1 mm). In the pig renal vein, small widely separated bundles (average 30 by 70 μ) of longitudinal fibers contain 8–25 fibers in cross-section. The muscle fibers of large arteries are mainly circular (or spiral) and occur in lamellar sheets widely separated by both collagenous and elastic connective tissues (fig. 1A).

Dimensions of individual fibers were measured from nitric-acid-glycerine maceration, from sectioned muscles by light microscopy and from electron micrographs. For each of these the muscles were fixed under similar conditions, i.e. at twice the 'rest' length. The length of individual fibers was longer for the fast conducting muscles, 220 μ for pig esophagus, 150 μ for taenia, 120 μ for both layers of cat intestine, 90 μ for dog retractor penis and 30 μ for pig carotid (table 1). The maximum diameters, measured in the region of the center of the nucleus, were remarkably similar, 5–7 μ for all except the arteries and veins which have 3-μ fibers. Because of longer fiber length, more small fibers are seen in cross-section of esophagus than of dog retractor penis. The longitudinal fibers of the pig renal vein were unusually long for blood vessel fibers (140 μ x 3.3 μ).

TABLE I

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Conduction Velocity, cm/sec</th>
<th>Cell Length, μ</th>
<th>Cell Diameter, μ</th>
<th>Extracellular Space, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig esophagus</td>
<td>220</td>
<td>6</td>
<td>12.0</td>
<td></td>
</tr>
<tr>
<td>Taenia coli</td>
<td>150</td>
<td>6</td>
<td>12.0</td>
<td></td>
</tr>
<tr>
<td>Dog retractor</td>
<td>No conduction</td>
<td>3</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Pig carotid</td>
<td>30</td>
<td>3</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Pig renal vein</td>
<td>110</td>
<td>3</td>
<td>30</td>
<td></td>
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</tbody>
</table>

m: muscle fibers; CT: connective tissue; ET: elastic tissue.
Cells of visceral muscles stretched to twice the usual fixation length were approximately twice as long; extension made the cells longer and thinner and there appeared to be little slippage. When arterial muscle length was increased, the cells became more closely packed, hence there may have been slippage.

Observations with the electron microscope. A general view of a typical smooth muscle, the cat circular intestinal muscle, is given in figure 2. Details of cell membranes, vesicles and intracellular details are shown in figures 3–7.

Separation of fibers; membranes. Calculations of extracellular space within bundles were made from many measurements from both transverse and longitudinal sections of each tissue. Electron micrographs of 10,000X magnification were enlarged four times and the areas of cells and of extracellular space were measured by means of a grid of 1-mm squares. The conditions of fixation were similar for all muscles with respect to pH and osmotic concentration; hence, such shrinkage as may have occurred should have been comparable for all muscles. Results are given in table 1. There was a graded correlation between conduction velocity and the extracellular space within bundles. The fast-conducting pig esophagus muscle had very little extracellular space, 4-4 %, the slower taenia coli and cat intestine 9.0–12.5 %, and the slow dog retractor penis 18.2 %, while the extracellular space in the pig carotid artery muscle in which no conduction occurs was 39 %. It is concluded that in the faster conducting muscles the fibers are more closely packed than in the slower or nonconducting muscles.

Many measurements of the spacing between muscle fibers were made at enlargements of 44,000 and 66,000X. When the average spacings were plotted against their frequency of occurrence, two peaks were seen for the slower muscles, one peak for the faster ones. The spacings (WS, wide space, and NS, narrow space) of the various muscles are compared in figure 3 and table 2.

The average separation for esophagus and the predominant separation for taenia was about 700 A; the separation in cat circular muscle was extremely variable, averaging 1200 A and in the cat longitudinal muscle, where no bundles occur, this space averaged 1600 A. The predominant spacing in the slow dog retractor penis was 3000 A. The average spacing for arterial muscle was about 10,000 A, which was much greater than that found in the visceral muscles.

The cell membrane consisted of a prominent granular outer or basement layer which varied in density in different regions. A light area separated this from the denser plasma membrane. A concentration of cytoplasmic vesicles was commonly seen beneath the dense plasma membrane (figs. 2 and 3).

No protoplasmic continuity was seen in any of the muscles. There were, however, two types of region of very close contact between adjacent cells without any discontinuity of cell membranes. Most commonly two cytoplasmic extensions from adjacent cells came into close contact (fig. 5A, B). Sometimes, especially in the pig esophagus, a protuberance of one cell was inserted into a pocket of an adjacent one (N, neck, fig. 5C). These ‘bridges’ (B) crossed by complete membranes were seen in muscles of intestine, taenia, esophagus and dog retractor penis, but not in blood vessel muscles. They were about 2500 A in diameter and usually 1000–2000 A long at the narrowest diameter. It is envisaged that in living muscle, the fiber surfaces are fluid and may make many pseudopod-like contacts between cells. At the points of closest contact between cells it is unlikely that much extracellular fluid space exists between the two apposed membranes. The taenia coli and cat intestinal fibers had rather smooth surfaces whereas those of the pig esophagus and dog retractor penis were more convoluted (figs. 3 and 6).

Intracellular structure. The most distinctive feature of the visceral smooth muscles as seen with the electron microscope was the abundance of vesicles (figs. 4, 5, 6). Two main types were distinguished (table 3). Large clear vesicles (V) with a wide range of size and shape (1000–4000 A), were arranged superficially in the fibers (fig. 5A). Most of these vesicles had a vacuolar appearance and some, connected by a neck to the extracellular space, were probably pinocytotic (PV) (figs. 4B, 7A and D). Small vesicles (V) of more specific size (100–300 A) were seen in large numbers, closely associated with the larger vacuole-type vesicles (fig. 5A, B, C). They were usually arranged in single or double layers beneath the

![Fig. 2.](http://ajplegacy.physiology.org/Downloadedfrom/http://ajplegacy.physiology.org/Downloadedfrom)
plasma membrane (fig. 6D). A mixture of large and small vesicles formed protruding longitudinal rows down the muscle fiber (figs. 2, 4A, 6B, 7E). There were 15–20 of these rows of vesicles per cell, the rows being about 0.5 \mu apart.

Vesicles of the same size as the small peripheral vesicles were sometimes visible deep in the cytoplasm (Re, fig. 6B, C and D). These cytoplasmic vesicles which were evenly spaced and uniform, may represent the endoplasmic reticulum.

Mitochondria were most abundant near the ends of the nucleus and behind the longitudinal strings of large vesicles (figs. 6B and 4A). The nuclei had clearly defined double membranes (fig. 4A).

Many thin parallel myofilaments (F), sometimes arranged in pairs, were found in the cytoplasm (figs. 2 and 4C). These were studied in sections of both fresh and glycerinated muscle. They showed no periodicities following osmic acid fixation. Extraction with alkaline 0.25 M KCl (pH 8.5) removed the myofilaments, hence they are presumably actomyosin. Also randomly dispersed in the cytoplasm were dark staining areas (DB, dark bodies) about 70 x 400 \mu in size (figs. 2, 3D and E, 4C). Myofilaments sometimes appeared to enter these bodies but at other times they were clearly separated from them and certainly did not converge on them. The dark bodies remained after extraction in alkaline KCl; they were not removed by extraction for 12 hours in KCl of various ionic strengths and pH up to 7.8. Cat intestinal muscle was treated at appropriate pH's with lipase, snake venom lecithinase, RNAase, hyaluronidase, lysozyme, trypsin and papain. None of these enzymes showed any preferential digestion of either the dark bodies or the surrounding matrix. Papain and trypsin removed the myofilaments first and after prolonged treatment the dark bodies as well. To test whether the dark bodies contain lipids, the material was dehydrated in an ethanol series, extracted with ethanol:ether: chloroform (2:2:1), dehydrated in the alcohol series and fixed with osmium for electron microscopy. Although the cell contents were completely disrupted, the dark bodies did not disappear. Since these structures

FIG. 3. Comparison of long sections of muscles with different spacings; A: cat longitudinal intestinal muscle; B: guinea-pig taenia coli; C: pig cartoid artery; D: pig esophagus muscularis mucosae; E: dog retractor penis.
remained after prolonged extraction in 50% glycerol, it is probable that they are not carbohydrate. It appears that the dark bodies consist of protein but not of contractile protein.

In examining grids of many sections one is struck by the paucity of nerve fibers (N, fig. 7D). The blood vessel muscles were characterized by enormous cell separations with much collagen and occasional striated muscle fibers in the intercellular spaces. The blood vessel cells branched considerably and had an ameboid appearance. No branching was noted in the visceral muscle fibers although they sometimes appeared frayed at the ends. Vesicles of the large vacuolar type were abundant in the blood vessel fiber cytoplasm.

DISCUSSION

The electron micrographs of gut muscles resemble those of uterus (2, 6) and bladder (7). The amount of
FIG. 5. Intercellular 'bridges' traversed by membranes; A and B: cat intestinal muscle; C: pig esophagus.

FIG. 6. Longitudinal sections showing undulating and straight membranes, rows of vesicles, vacuoles and mitochondria; A and D: pig esophagus; B and C: cat longitudinal intestinal muscle.
CONDUCTION IN SMOOTH MUSCLE

FIG. 7. Cross sections to show marginal vesicle; A: retractor penis at high magnification; B: renal vein; D: dog retractor penis; C and E: cat longitudinal intestinal muscle at two magnifications.

TABLE 2. Intercellular Spacings in Angstrom Units

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Narrow Spacings</th>
<th>Wide Spacings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig esophagus (muscularis mucosae)</td>
<td>756±41 (383-1796)</td>
<td>380±g (297-1140)</td>
</tr>
<tr>
<td>Guinea-pig taenia coli</td>
<td>*607±27 (274-1276)</td>
<td>290±g (297-1140)</td>
</tr>
<tr>
<td>Cat circular intestinal</td>
<td>1,247±5.8 (350-2621)</td>
<td>*492±360 (3554-6975)</td>
</tr>
<tr>
<td>Cat longitudinal intestinal</td>
<td>*1,640±177 (756-3310)</td>
<td>409±360 (3554-6975)</td>
</tr>
<tr>
<td>Dog retractor penis</td>
<td>1,058±169 (649-2425)</td>
<td>*3960±390 (2465-7215)</td>
</tr>
<tr>
<td>Pig arteries</td>
<td>10,419±1475 (4,152-18,845)</td>
<td>*3960±390 (2465-7215)</td>
</tr>
</tbody>
</table>

Values are means ± standard error; figures in parentheses give range. * Predominant spacing.

extracellular space obtained morphologically agrees well with the inulin space of cat intestinal muscle from which most nonmuscular tissue had been removed (8). There is considerable variation in amount of connective tissue present between fibers in the different muscles. The intercellular spacing correlates well with conduction velocity and electrical resistance (9). The large vesicles have been seen previously in visceral muscle, although not noted in longitudinal rows. Many of these may be pinocytotic vesicles (7) and are indicative of high permeation activity. The small deeply placed vesicles may or may not be part of an endoplasmic reticulum.

Contractile protein seems to be well localized in myofilaments which are random but parallel. The dark bodies have been shown in other smooth muscles (7). On the basis of the numerous chemical treatments used, it is only possible to conclude that they consist of noncontractile protein. Localization of tropomyosin was not obtained and the extraction methods clearly establish that no paramyosin-like protein is present in visceral smooth muscle (10, 10a).

None of the muscles examined showed any evidence of protoplasmic continuity, such as reported (2) for uterus and (11) for stomach of young rats. Cell protruberances in intimate contact form bridges crossed by continuous membranes of adjacent cells as described by Bergman (12). With less shrinkage in the present material, the length of the bridges is less than Bergman’s. These bridges are 2500 Å in diameter and 1000–2000 Å long in the narrowest regions. Even if they had no membranes (like those figured in (11)) each bridge would have a resistance of about $10^8$ ohms; with membranes they would probably have an even higher resistance. Thus it
is improbable that the bridges constitute really low resistance paths between the fibers. However, if the resistance of a bridge is lower than, or even equal to, the total membrane resistance across adjacent cells, potentials would be divided in such a way that current from spike responses could flow through adjacent cells as well as between them, i.e. it is a relative rather than an absolute low resistance pathway which is the significant requirement for conduction. The resistance between the cell interior and the extracellular medium was measured to be about 100 megohms (13). It may well be that the specific resistance of the membranes across a bridge is less than that of membrane in other parts of the cell, or that the resistance drops during activity.

Another possible function of the bridges might be mechanical, to hold the muscle fibers together. The bridges may be permanent structures or if the cell membranes are continually varying in contact in the living state, they may represent points of contact at the instant of fixation. Similar contacts between cells have been frequently observed in tissue cultures of epithelial cells and even in liver (14) where they are assumed to have a mechanical function.

Correlations between membrane morphology and electrical properties will be suggested in a succeeding paper.

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REFERENCES