1-1-2009

Unique Contractile and Structural Protein Expression in Dog Ileal Inner Circular Smooth Muscle

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Published version. *Journal of Smooth Muscle Research*, Vol. 45, No. 5 (2009): 217-230. DOI.

Unique contractile and structural protein expression in dog ileal inner circular smooth muscle

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Received July 27, 2009; Accepted August 17, 2009

Abstract

This study was designed to test the hypothesis that there is heterogeneous expression of contractile and structural proteins between the smooth muscle cells (SMCs) in the inner and outer circular muscle (ICM and OCM) layers of the ileum. Immunohistochemical staining and quantitation of fresh frozen sections of the dog ileum was performed using protein specific antibodies. Smooth muscle (SM) SMA myosin heavy chain (MHC), α- and γ-SM actin, and vinculin all show greater expression in the ICM relative to the OCM. SMB MHC and fibronectin show the opposite pattern, with greater expression in the OCM relative to the ICM. Differences in expression of these proteins are consistent with proposed differences in function of these muscle layers. Hypotheses regarding muscle tone and the coordination and regulation of peristalsis via these different muscle layers based on this data can now be made and tested.

Key words: contractile proteins, cytoskeletal proteins, small intestine, ileum, digestion

Introduction

The gastrointestinal system is very complex anatomically and physiologically. The small intestine is composed of a number of layers of tissue, including the mucosa, muscularis mucosa, submucosa, muscularis externa (circular and longitudinal muscle layers) and serosa. The circular muscle layer has been described as having a distinct inner layer of smooth muscle cells (SMCs) in guinea pig, mouse, rat, rabbit, cat, dog, and human (Li, 1940; Duchon *et al.*, 1973; Gabella, 1974). In the dog, this inner circular muscle (ICM) layer of cells is often 6–12 cells thick (Zelcer and Daniel, 1979). These ICM cells are smaller, and lack gap junctions even though their intercellular space is smaller than the outer circular muscle (OCM) cells (Duchon *et al.*, 1973; Zelcer and Daniel, 1979). These morphological differences may be indicative of physiological differences between these two regions of the circular muscle layer.

We have observed that SMCs from various smooth muscle (SM) organs heterogeneously express different amounts of the myosin isoforms (Meer and Eddinger, 1996; Eddinger *et al.*,

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2000; Eddinger and Meer, 2001; Parisi and Eddinger, 2002). Myosin isoform content may be particularly important to smooth muscle physiology because isoform composition is known to be the principle determinant of contractile characteristics in other contractile cells. Tonic SM slowly develop sustained tone when activated, whereas phasic SM rapidly develop transient tone (Somlyo and Somlyo, 1968). SM from tissues such as the stomach fundus, aorta and other large vessels and sphincters are characterized as tonic muscle, where slow sustained contraction is needed for optimal organ function. Conversely, stomach antrum, gizzard, most of the gastrointestinal SM, and esophageal body (when it is SM) are primarily phasic muscles, where frequent comparatively rapid contraction may be required for proper organ function. Special attention has recently focused on the 25–50 kDa junction domain of myosin S1 head, the region of the MHC ATP-ase activity. This is where the 7 amino acid insert resides and appears to be correlated with differences between tonic and phasic contraction (Hamada *et al.*, 1990; Kelley *et al.*, 1993; Rovner *et al.*, 1997; Eddinger and Meer, 2001). Reported possible physiological implications for the SM MHC tail (SM1 and SM2), $MLC_{17a\&b}$, and the non-muscle (NM) MHC (NMA, B, & C) isoforms in SM contraction include thick filament formation and stability and latch bridge formation, but there is much less agreement about these roles (see Eddinger and Meer (2007) for review).

Because myosin is known to interact with actin to generate force and or cause cell shortening, expression and regulation of the actin isoforms is also of interest. There are six different actin isoforms that can be present in vertebrates (Garrels and Gibson, 1976; Vandekerckhove and Weber, 1978) that are the product of six separate genes (Firtel, 1981; Chang *et al.*, 1984) Currently there is no agreement or direct evidence that the actin isoforms have unique function. Ultimately, the forces generated by myosin and actin need to be transmitted to the cell membrane and to neighboring cells. Vinculin (cytoskeletal) and fibronectin (extracellular) are involved in transmitting the force generated by the contractile apparatus to the cell membrane (integrins) and from one SMC to another. In this way the SMCs form a mechanical syncitium where the contractile force generated in one cell may be transmitted to neighboring cells.

This study used immunological techniques to define the expression and distribution of several contractile and structural proteins in the dog ileum to support the hypothesis of unique function in the ICM relative to the OCM. Differences in contractile and structural protein expression between the ICM and the OCM add to the list of other known differences between these SM layers. Because there is a strong correlation between the expression of the SMB *vs.* SMA MHC in "phasic" *vs.* "tonic" mechanical function in SM tissues and cells (Eddinger and Meer, 2007), and their increased expression in the OCM and ICM respectively, we hypothesize that the ICM is primarily involved in maintaining basal tone in the ileum, while the OCM is primarily involved in "phasic" contractions involved with peristalsis. The results of this study are consistent with this hypothesis.

Materials and Methods

Organ handling and cell isolation

Experimental procedures were approved by the Institutional Animal Care and Use Committees of the Medical College of Wisconsin and the Zablocki Veteran Affairs Medical Center (dogs), and Marquette University (rabbits). Swine stomach tissue was obtained from Harry Hansen Meat Service (Franksville, WI). Organs (ileum and stomach) were harvested from euthanized dogs (overdose of anesthetic and KCl) used at the VA Medical Center following acute vascular studies (performed on the rear leg) and put in cold physiological salt solution (PSS; (in mM): 140.1 NaCl, 4.7 KCl, 1.2 Na_2HPO_4, 2.0 MOPS (pH 7.4), 0.02 Na_2EDTA, 1.2 $MgSO_4$, 1.6 $CaCl_2$, and 5.6 glucose). Ileum was cleaned of chyme, blood, loose connective tissue, and frozen in isopentane cooled in liquid nitrogen, and stored at –20°C until serially sectioned. Rabbit stomachs were removed following CO_2 asphyxiation. SM from the antrum and fundus of swine, dog and rabbit was isolated and ground in sample buffer for western blotting.

Immunoreactions

The antibodies used were obtained from the following sources: generic (total) actin (A4700), α-SM actin (A2547), β-NM actin (A5441), fibronectin (IST-3), and vinculin (VIN-11-5), from Sigma Chemical Co, St Louis MO; γ -SM actin (clone B4) from MP Biomedicals, Aurora, OH; Cy2 and Cy3 labeled donkey anti mouse, rat, or rabbit secondary antibodies - Jackson ImmunoResearch, West Grove, PA; Alexa Fluor 594-phalloidin and DAPI from Molecular Probes, Eugene, OR. Antibodies were tried at concentrations over several orders of magnitude bracketing their recommended use concentrations to optimize the signal to noise ratio.

Polyclonal antibodies to SMA MHC were generated using the rabbit vascular myosin head insert sequence without the extra 7 amino acids ('N'—KKDTSITGELEC—'C') and to SMB MHC by using the rabbit visceral myosin head insert sequence with the extra 7 amino acids ('N'—QGPSLAYGELEC—'C') as antigens (Babij, 1993). The peptides were conjugated to Keyhole Limpet Hemocyanin using a malemide activation kit (Pierce, Rockford, IL). Pre-immune serum was collected from rabbits prior to the first injections. Rabbits were injected at multiple sites along their back with approximately 0.25 mg of conjugated peptide mixed with Freunds complete adjuvant and were given booster shots at approximately 1 month intervals. Blood was collected for antibody purification at 2 week intervals starting one week after the first booster injection. Sodium azide (0.02%) was added to the isolated serum which was stored frozen. Both the SMA and SMB MHC antibodies were found to have an approximately 100- fold higher specificity for their respective MHC isoform.

Five micron thick frozen tissue sections were picked up on glass slides, air dried, fixed with 2% paraformaldehyde for 10 min, permeabilized in 0.5% Triton X-100 for 10 min and blocked with 5 mg/ml BSA for 1 hour prior to reacting with the primary antibody for one hour and then the appropriate secondary antibody for one hour at room temperature. After the secondary antibody, the tissues were incubated with DAPI (0.5 μM), phalloidin (10–50 nM) or DAPI/ phalloidin as appropriate for staining nuclei and/or filamentous actin respectively. Multiple

washes with PBS-tween (in g/liter: NaCl 8.0, KH_2PO_4 0.2, Na_2HPO_4 1.15, KCl 0.2, 1% tween-20, pH 7.4 with 0.1% BSA) were used following the primary and secondary incubations. Cover glass was mounted using buffered 75% glycerol with 0.2% n-propyl gallate to minimize fading and sealed with clear nail polish before being stored at –20°C until being examined. Negative controls included leaving out the primary antibody or using serum in place of the primary antibody.

Microscopy and quantitation

Sections were observed using an Olympus IX70 microscope with epifluorescence illumination. Digital images were taken with a 16bit Princeton Instruments (Princeton, NJ) CCD camera, controlled through a PCI board via IPLab for Windows on a PC (Ver. 3.6, Scanalytics; Fairfax, VA). Immunofluorescence images were taken using either a 60× (1.25 NA) oil lens or a 40× (0.9 NA) or 20× (0.4 NA) air lens and stored on the PC. Emission filters used were 405, 490 and 570 nm. Montages were assembled in Adobe Photo Shop (6.0, Adobe Systems Inc., San Jose, CA). Tissues from three to 5 animals were used for each antibody. Immunofluorescence images were used to quantify the levels of fluorescence in the inner and outer circular muscle layers. For each tissue section and each antibody used, five identically sized defined regions of interest (ROI) were delineated in both the ICM and adjacent OCM. The regions were selected to be non-overlapping and to be completely inside the SM layer of either of these regions of the circular muscle. The total fluorescence signal from each of the five regions was averaged to give a single "n" for that antibody. A two-tailed *t*-test was done for each antibody with a P value<0.05 indicating that the level of fluorescence was significantly different between these two layers. Normalizing the fluorescence signal of each antibody to the phalloidin counter stain fluorescence signal for the same ROI's gave the same results. In addition, because all of the comparisons were made on adjacent regions of the circular muscle from the same section on the same slide, all conditions were similar in the handling and processing, removing any possible differences in processing.

Polyacrylamide gel electrophoresis, western blotting and ELISAs

MHC isoform expression was analyzed as described previously (Han *et al.*, 2006). SM tissue from the stomach antrum and fundus were homogenized in 0.125 M Tris, 2% sodium dodecylsulfate (wt/vol), 20% glycerol, 0. 1% bromophenol blue (wt/vol) and 20 mM dithiothreitol. MHCs were resolved on low cross-linking sodium dodecylsulfate gels (Giulian *et al.*, 1983) and transferred to nitrocellulose for immunoblotting as previously described (Eddinger and Wolf, 1993). Expressed SMA and SMB HMM (Eddinger *et al.*, 2007) was a gift of AS Rovner and was used for the ELISA assay, along with western blotting, to test for SMA/B specificity.

Results

Antibodies specific for the SMA/B MHC isoforms (without and with the 7 amino acid head insert, respectively; see methods for sequences) were generated in rabbits and tested on

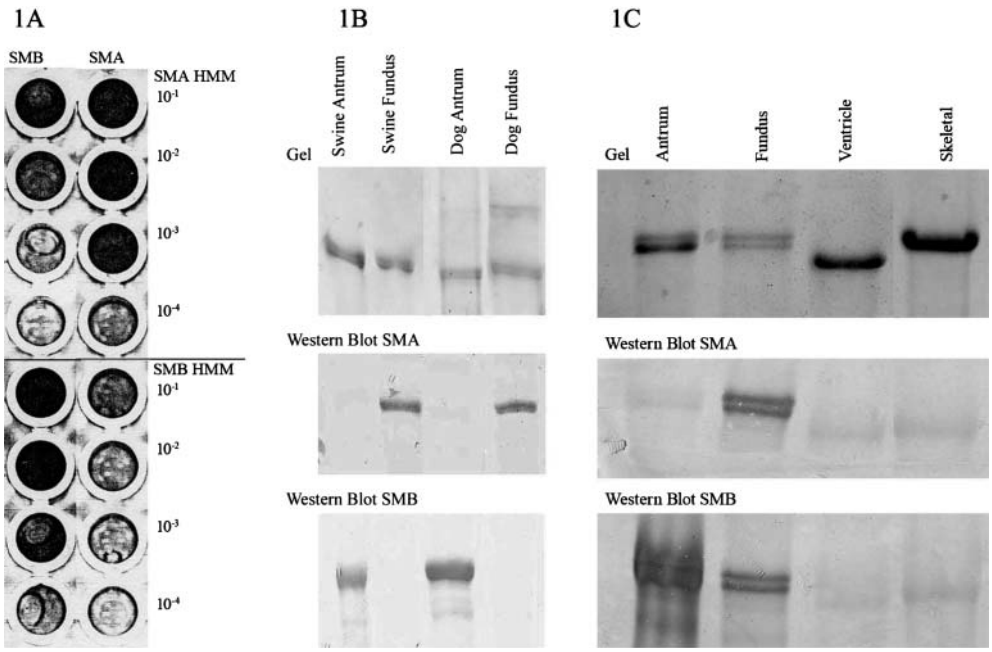

Fig. 1. 1A. ELISA showing immunoreactivity of the rabbit polyclonal SMA and SMB MHC antibody for expressed SMA and SMB HMM (serial dilution of expressed SMA/B HMM from 10^{-1} to 10^{-4}). The SMA antibody recognizes the SMA HMM at ~100-fold lower concentration than the SMB HMM. The converse is true for the SMB MHC antibody. 1B. Gel and blots of identical gels showing the MHC composition of swine and dog antrum and fundus stomach smooth muscle. MHC doublet results from size difference between the SM1/2 isoforms (~204 and 200 kD respectively). The blots show that the SMA MHC antibody reacts strongly with the fundus SM while the SMB MHC antibody reacts strongly with the antrum SM. 1C. Gel and blots of identical gels showing the MHC composition of rabbit antrum (~95% SMB MHC), fundus (~80% SMA MHC), ventricle and skeletal muscle. The SMA MHC antibody reacts strongly with only the rabbit fundus SM while the SMB MHC antibody reacts strongly with only the rabbit antrum SM.

expressed SMA/B HMM and smooth muscle (SM) tissues. ELISA results showed that each antibody has an approximately 100-fold higher specificity for its respective antigen (Fig. 1A). Gel electrophoresis resolves the SM1/2 MHC isoforms because of their size difference (34 AA, SM1 upper and SM2 lower), but does not resolve the SMA/B MHC isoforms (7 AA). Both the SM1 and SM2 isoforms can be either SMA or SMB, which are not resolved by the gel and must be identified by western blots. Western blots of SM tissue from stomach fundus and antrum (primarily SMA and SMB MHC respectively; Eddinger and Meer, 2001) are preferentially labeled with the SMA and SMB antibodies (Fig. 1B). Neither of these antibodies showed significant reactivity with cardiac (ventricle) or skeletal (hind limb) muscle tissue (Fig. 1C), consistent with previously reported expression of MHC in these organs. Rabbit fundus is approximately 20% SMB MHC explaining the reactivity of both the SMB and SMA antibody to fundus myosin.

The ileum muscularis externa is composed of the longitudinal and the circular muscle layers. The circular muscle has been described to consist of the inner circular muscle and the

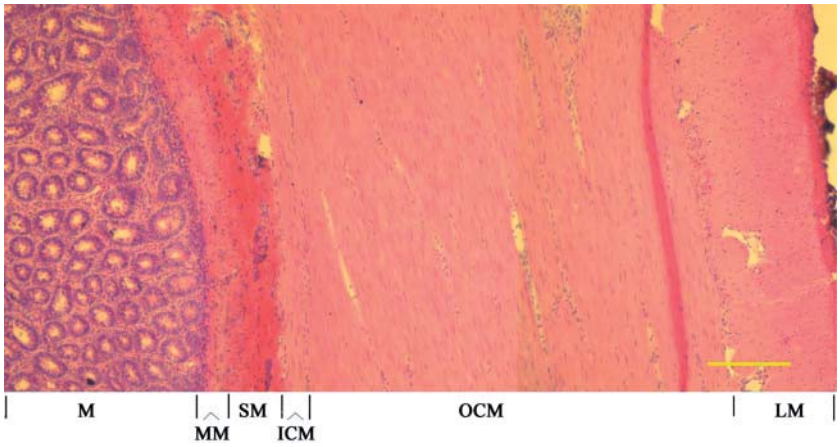

Fig. 2. Transverse H&E section through dog ileum. From left to right, and in all following immunohistochemical sections, the mucosa (M), muscularis mucosa (MM), submucosa (SM), circular smooth muscle layer (ICM- inner circular muscle and OCM- outer circular muscle), and the longitudinal layer (LM, this figure only). Note the location of the ICM relative to the OCM, the difference in nuclei density, and the chain of nuclei that separates the ICM from the OCM. Scale bar equals 100 μm.

outer circular muscle, which are separated from each other by the deep musclar plexus or the plexus muscularis profundus (Fig. 2). The ICM is composed of SMCs that are anatomically smaller than those from the OCM.

Immunohistochemistry was used to determine the distribution of several major contractile and structural proteins that are purported to be involved in SM function and be uniquely expressed in different SM tissues. Figure 3 shows a negative control for the secondary antibodies used in this work. The background fluorescence in negative control samples was significantly below the fluorescence obtained for any of the proteins examined in this study using the same exposure conditions. Figure 3 also shows that both the phalloidin staining for filamentous actin and the "total" actin antibody give uniform staining across the ICM and OCM. Thus the differences observed between these layers using the other antibodies are not an artifact of differential reactivity between these regions of the ileum. The ICM shows significantly higher expression than the OCM for the SMA MHC while the SMB MHC expression is significantly lower in the ICM relative to the OCM (Fig. 4).

The actin isoforms were also examined as they too can show tissue specificity. α-SM actin showed the most dramatic difference in expression between the ICM and OCM layers, with the ICM showing significantly greater expression for the α-SM actin isoform (Fig. 5). The γ-SM actin isoform also showed significantly greater expression in the ICM over the OCM (Fig. 5), but not as varied as the α-SM actin. The β-NM actin expression was very low in the ICM and OCM (Fig. 5).

Structural proteins directly (or indirectly) involved in transmitting force to the membrane and in cell-cell attachment also showed variable expression between the ICM and OCM. Fibronectin shows a significantly lower level of expression in the ICM relative to the OCM while

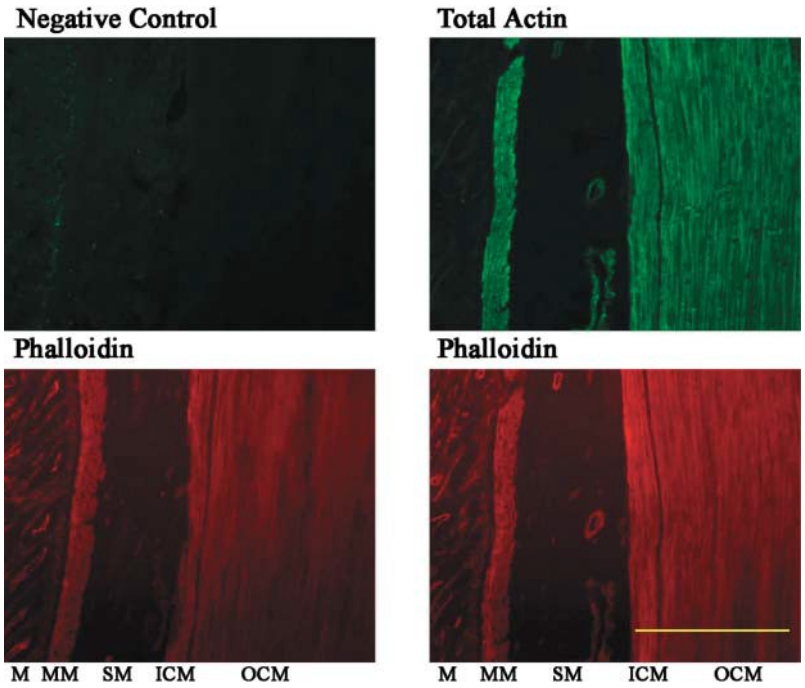

Fig. 3. Dog ileum immunohistochemical negative control (left) and total actin immunoreactivity (right). Dog ileal transverse section showing from the mucosa to the outer circular muscle layer (see Fig. 2 for labels). No primary antibody was used (left upper panel) while a total actin primary antibody was used in the upper right (Cy2 exposure-green). Lowers panels are the same sections as the top panels showing the Cy3 (red) exposure for phalloidin. Antibody labeling times and picture exposures were all kept constant for control and antibody labeled sections. Note that the total actin antibody (upper right) and the phalloidin counter stain (lower panels) both show a uniform immunoreactivity throughout the SM layers shown (MM, ICM and OCM). Scale bar equals 100 μm.

vinculin shows a significantly stronger expression in the ICM relative to the OCM (Fig. 6). Both of these proteins are located at the cell periphery (fibronectin extracellularly and vinculin intracellularly). A summary of the quantitative results with the *P* value for significance for all of these proteins is given in Table 1. With the exception of the β-NM actin and the total actin expression, all the other proteins examined showed a significant difference in expression between the ICM and OCM (Table 1).

Discussion

The presence of two distinct anatomical regions of the circular muscle layer of the muscularis externa in the small intestine has been known for some time. Li (1940) stated "…the circular muscle of the small intestine of various vertebrates is subdivided into two layers, a thin inner layer and a thick outer layer. The inner layer is composed of one to several layers of muscle cells which are much smaller in size, sometimes branching and anastomosing with other

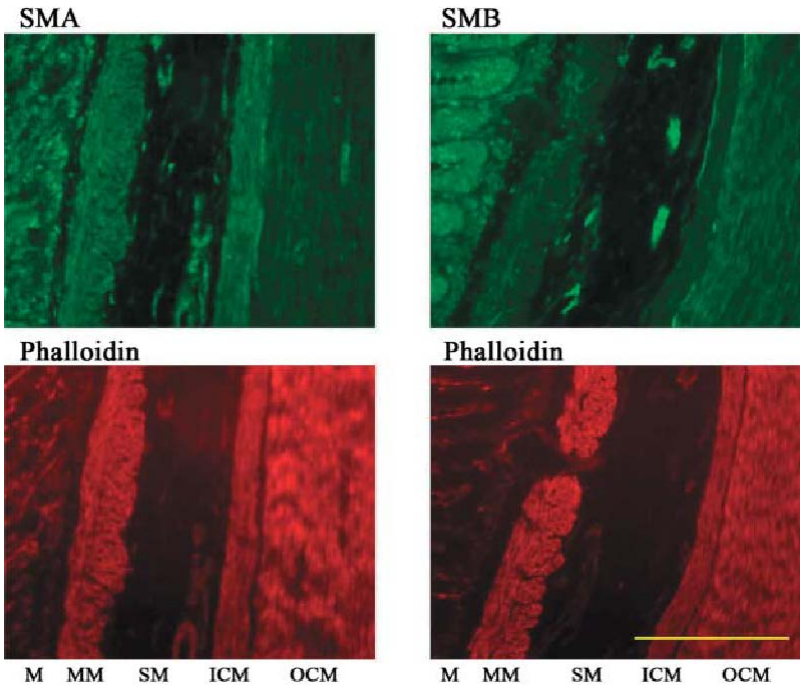

Fig. 4. SMA (left) and SMB (right) MHC immunoreactivity in dog ileum. Upper and lower panels show the same section with exposure at different excitation and emission wavelengths. Upper panels show MHC immunoreactivity with phalloidin counterstaining in the lower panels. Phalloidin shows relatively uniform staining across all SM tissue layers. Note the strong immunoreactivity of the SMA MHC antibody in the ICM relative to the OCM. The SMB MHC antibody shows reduced immunoreactivity in the ICM relative to the OCM. See Fig. 2 for labels. Scale bar equals 100 μm.

muscle cells. This layer is richer in nuclei than the outer layer." She hypothesized that this layer may be "neuromuscular" and account for the "irritability or conduction" of the small intestine. Since that time, numerous other papers have noted that there are two anatomically distinct regions of the small intestine circular muscle layer in various species. Duchon *et al.* (1973) reported a "distinct layer of circular muscle, adjacent to the submucosa" with cells that are smaller and more closely packed than the main region of circular muscle cells in rabbit, dogs and humans. This inner layer is 6–12 cells thick, and isolated from the rest of the circular layer by nerve axons and connective tissue. They reported that these layers differ in: 1) larger intercellular spaces between the larger SMCs with numerous gap junctions (nexus) in the OCM layer and smaller intercellular spaces and smaller SMCs with no gap junctions in the ICM layer; 2) numerous nerve axons in the OCM and few if any in the ICM; 3) SMCs in the OCM have nexus with the plexus muscularis profudus (deep musclar plexus) while the SMCs in the ICM do not. They hypothesized that this lack of nexal connections may inhibit action potential propagation between these ICM cells and this could result in independent contractions between these two layers. Gabella (1974) reported the presence of "small dark cells" at the luminal side of the circular muscle layer in mice, rats, guinea pigs, rabbits, sheep, cats, and dogs that are not

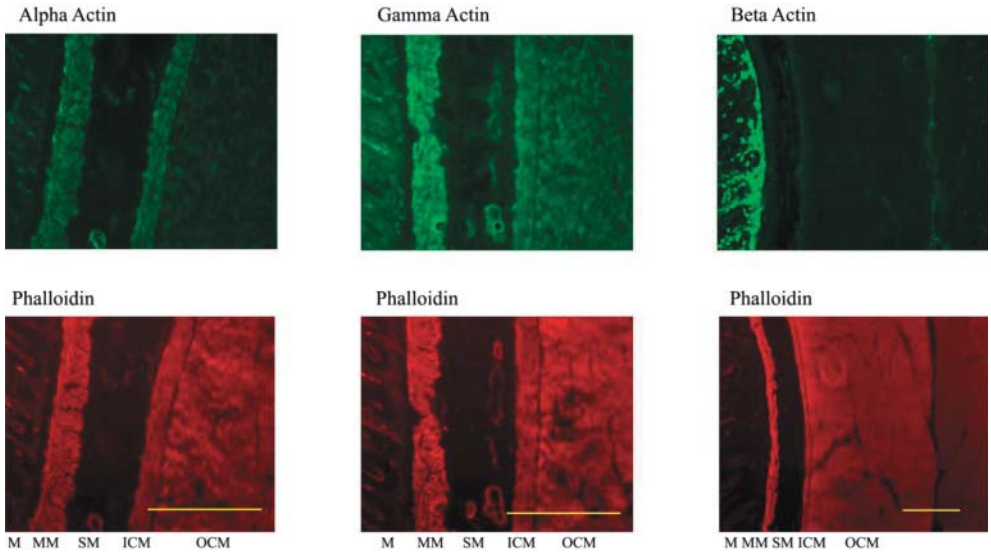

Fig. 5. Portion of transverse section from dog ileum. Upper panels show α-SM actin (left), γ -SM actin (middle) and β-NMactin (right) immunoreactivity with phalloidin immunoreactivity (filamentous actin staining) in the lower panels directly below. Phalloidin shows relatively uniform staining across all SM tissue layers. Note the α- and γ - SM actin immunoreactivity is significantly stronger in the ICM *vs.* the OCM while the β-NM actin immunoreactivity is very low in all circular SMCs. See Fig. 2 for labels. Scale bar equals 100 μm.

observed in the stomach, colon or rectum. He also failed to find nexus between these "small dark cells". Gabella (1974) hypothesized that because of their location and lack of connectivity, the ICM could be a "pressure and/or volume" sensor and "would be an advantageous position for a stretch-sensitive apparatus, whose initial length could be set at any point over a wide range by means of fine motor control".

There have been numerous publications mentioning these two distinct regions of the circular muscle layer more recently, but usually in the context of the deep muscle plexus that divides these regions and the role of interstitial cells of Cajal (ICC) in regulating smooth muscle contractile activity (Zelcer and Daniel, 1979; Thuneberg, 1982; Faussone Pellegrini and Cortesini, 1983; Faussone Pellegrini, 1984; Gabella, 1984; Rumessen *et al.*, 1993; Torihashi *et al.*, 1993; Thuneberg *et al.*, 1995; Burns *et al.*, 1997; Thuneberg and Peters, 2001). Faussone Pellegrini (1984) looked at the morphogenesis of the ICM in mouse and reported that the fetus at term is lacking both the ICM and the longitudinal muscle layer. The ICM is first visualized in the neonate with its development lagging behind the OCM. It is not until post-natal day 30 that the ICM is fully differentiated. Based on the matching developmental time course of the ICM, plexus muscularis profundus and ICC, Faussone Pellegrini (1984) proposed that these components are an "anatomical and functional unit".

We hypothesize that the ICM is in fact SMCs (presence of significant SM actin and SM myosin) and primarily involved in maintaining a basal tone in the ileum, while the OCM is primarily involved in "phasic" contractions involved with peristalsis. While lacking any direct

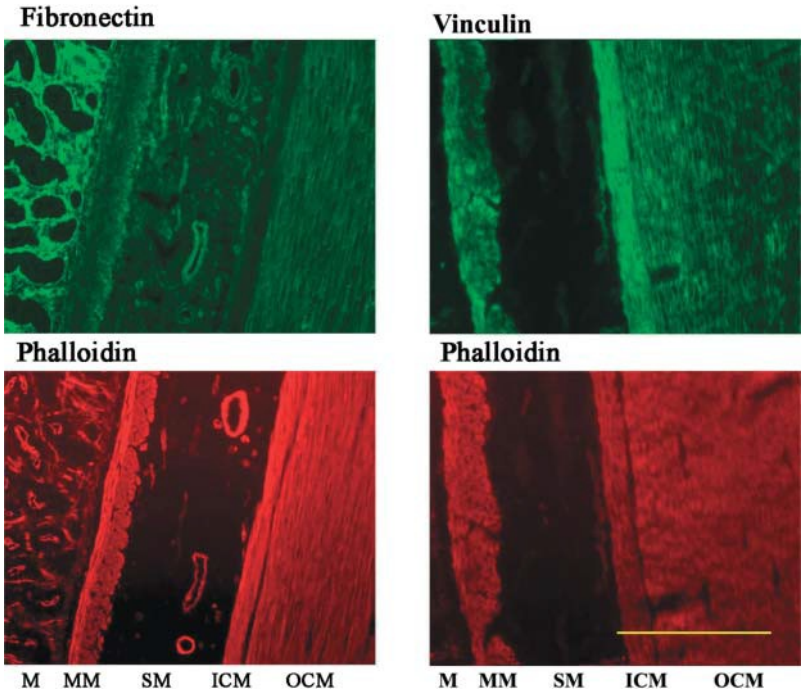

Fig. 6. Fibronectin (left) and vinculin (right) immunoreactivity in dog ileum. Upper and lower panels show the same section using different excitation and emission wavelengths. Phalloidin shows relatively uniform staining across all SM tissue layers. Note the weak immunoreactivity of the fibronectin antibody in the ICM relative to the OCM and the strong immunoreactivity of the vinculin antibody in the ICM relative to the OCM. See Fig. 2 for labels. Scale bar equals 100 μm.

Table 1. Protein distribution in inner (ICM) and outer circular smooth muscle (OCM) layer of the dog ileum

Protein	N	Distribution	*P* value
SMA	5	ICM>OCM	0.033
SMB	3	OCM>ICM	0.001
α-actin	4	ICM>OCM	0.010
β -actin	3	NS	0.278
γ -actin	3	ICM>OCM	0.042
Total Actin	3	NS	0.464
Fibronectin	3	OCM>ICM	0.046
Vinculin	4	ICM>OCM	0.042

support for this hypothesis, there is precedence for this in the stomach. The stomach is traditionally divided into three regions, the fundus, body, and antrum. Histologically, all three stomach regions are composed of two separate discernable muscle layers, the muscularis mucosa and the more massive muscularis externa (Weinshelbaum, 1974; Weisbrodt, 1974; Schultz *et al.*, 1989). Although there is no reported contractile difference we are aware of between stomach muscle layers in a given region of the stomach, stomach motility changes as

one moves along the greater curvature of the stomach (Weisbrodt, 1974; Schultz *et al.*, 1989). In general, tonic contractions are localized in the fundus of the stomach, which regulates the reservoir functions of the stomach and adjusts the overall size of the stomach as it pushes food to the antrum (Schultz *et al.*, 1989). Phasic contraction is localized to the antrum, where the stomach contents are mixed and ground before being passed to the duodenum (Schultz *et al.*, 1989). The body, the region between fundus and antrum, has been found to be a region in transition between antral and fundic extremes (Weisbrodt, 1974; Gabella, 1987; Schultz *et al.*, 1989). We have reported that there is strong positive correlation between the phasic antrum with high SMB MHC expression and the tonic fundus with low SMB MHC expression (Eddinger and Meer, 2001; Parisi and Eddinger, 2002). Thus, the presence or absence of the SMA/B MHC isoforms appears to correlate well with tonic and phasic contractile properties respectively. The non-muscle MHC isoforms can also be expressed in adult SM tissues but at levels significantly below the SM MHCs, and their possible role in SM contraction is controversial (see Eddinger and Meer (2007) for review).

The greater expression observed for the cytoskeletal protein vinculin in the ICM, may be a function of the smaller size of the SMCs in the ICM relative to the OCM. Smaller cells have a higher surface area to volume ratio, and thus membrane associated proteins would appear denser as the surface area to volume ratio increased. Functionally, the greater surface area to volume ratio and number of adherens junctions per unit area would also allow for stronger adhesion between these cells. In contrast, the reduced expression of the extracellular matrix protein fibronectin in the ICM may result from these SMCs being more tightly packed leaving less extracellular space for fibronectin, and/or the predominant use of other extracellular proteins in this region (laminin, collagen).

Further work is needed to examine the mechanical properties of these different populations of cells. The positioning of interstitial cells of Cajal (ICC) in the deep muscular plexus between the ICM and OCM layers may also be important in regulating these two regions of circular SM. Peristalsis is a complex coordination of contraction in the muscularis externa (circular and longitudinal layers) resulting in the mixing and aboral propulsion of foodstuffs through the intestinal tract that is believed to be regulated in part by the ICC (Huizinga, 1999; Rumessen and Vanderwinden, 2003; Sanders *et al.*, 2006). Liu *et al.* (1997) reported that there is no communication between SMCs in the circular lamellae of dog in the absence of ICC or branching SMCs. Because the ICM lacks gap junctions and connections to the ICC cells in the deep muscular plexus, and the absence of ICC in this layer (Thuneberg *et al.*, 1995; Rumessen and Vanderwinden, 2003), the ICM is functionally isolated from the rest of the circular muscle. In rat however, there is at least one report of gap junctions between the ICC in the deep muscular plexus and the SMCs of the ICM (Komuro and Seki, 1995).

The results of this study indicate that there is a significant difference in the expression of numerous contractile and structural proteins in the ICM and OCM layers. SMA MHC, α- and γ-SM actin, and vinculin all show greater expression in the ICM relative to the OCM. SMB MHC and fibronectin show the opposite pattern, with greater expression in the OCM relative to the ICM. No differences in expression were observed with the total actin or β-NM specific actin antibodies or with phalloidin staining for filamentous actin. These results, along with previously

published data showing morphological and electrophysiological differences between these cells leads to the hypothesis that the inner and outer circular SM cells have unique physiological function in GI peristalsis. Consistent with this data, we hypothesize that the inner circular SM cells are involved primarily in maintaining basal tone in the intestine while the outer circular SM cells are involved primarily in peristalsis. Using only a small subset of circular SMCs (ICM) to maintain tone would provide a very economical means of preventing ileal distension when there is little pressure on the muscle wall. The more massive OCM layer could be recruited during peristalsis and aboral propagation of chyme into the large intestine. Further work needs to be done to test this hypothesis.

Acknowledgements

This work was supported by the National Heart, Lung, and Blood Institute Grant R01-HL-62237. I would like to thank PS Clifford for providing dog tissues and Harry Hansen Meat Service for the swine tissue, AS Rovner for the expressed SMA and SMB HMM, and JA Parisi, JD Schiebout, DM Bizub, and L An for contributions to the immunohistochemistry, photo acquisition, data analysis, and figure preparation, and DR Swartz for comments on the manuscript.

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