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Smooth Muscle Spontaneous Activity

Physiological and Pathological Modulation

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Preface

Over the last 50 years, there have been a series of books published on *Smooth Muscle* including *Smooth Muscle* (Bülbring et al. 1970); *The Physiology of Smooth Muscle* (Bülbring and Shuba 1976); *Smooth Muscle: An Assessment of Current Knowledge* (Bülbring et al. 1981); *Frontiers in Smooth Muscle Research* (Sperelakis and Wood 1989) and *Smooth Muscle Excitation* (Bolton and Tomita 1996). The early books were reviews written by research scientists that had an association with one of the pioneers of smooth muscle physiology, Professor Edith Bülbring, and the Department of Pharmacology, Oxford University, while the latter two books arose after international gatherings that recognised the distinguished contributions to smooth muscle physiology of Professor Emil Bolzer as well as the retirement of Professor Tadao Tomita.

Just as important, these books reflexed the application of the most up-to-date technology to smooth muscle research. *Smooth Muscle* (1970) and *The Physiology of Smooth Muscle* (1976) reviewed existing knowledge of the structure and innervation of smooth muscle, the acceptance of membrane potential recordings with 'sharp' microelectrodes, the ionic basis of the action potential, the interpretation of electrical waveforms using cable analysis of syncytia and their pharmacological modification upon drug application or nerve stimulation. *Smooth Muscle: An Assessment of Current Knowledge* (1981) expanded this ultrastructural and electrophysiological knowledge into investigations into the mechanisms of calcium control and agonist modulation of contraction. *Frontiers in Smooth Muscle Research* (1989) and *Smooth Muscle Excitation* (1996), arising from international meetings, tended to be more experimental rather than review in nature and somewhat broader in scope. *Frontiers in Smooth Muscle Research* (1989) focused more on the biochemistry of smooth muscle, including the mechanisms of excitation-contraction coupling, energetics, biochemistry of contractile proteins, ion pumping and IP_3 metabolism, while *Smooth Muscle Excitation* (1996) detailed the most recent findings upon the application of patch clamp technology and the fluorescent imaging of calcium to unravel the role of calcium stores, their mechanisms of release and influence on contraction and the membrane ion channels underlying the electrical activity, as well as the recognition that cells other than smooth muscle cells may be driving spontaneous contraction.

Our book was conceived to again present more extensive reviews of the major smooth muscles currently under study: airways, phasic and tonic

gastrointestinal muscles, upper and lower urinary tracts, various reproductive organs and vessels of the vasculature and lymphatics.

What is clear from the reviews herein is that there are very few smooth muscle syncytia that are in fact 'homocellular' and 'myogenic', as thought in the 1960s. Over the last 25 years, it has become evident that many smooth muscle organs contain syncytia that are 'heterocellular': smooth muscle and interstitial cells in the oviduct, prostate and urethra; the 'SIP' syncytium in the gastrointestinal tract; atypical/typical smooth muscle and interstitial cells in the renal pelvis; mucosa, interstitial and smooth muscle cells in the bladder and seminal vessels; endothelium and mural cells, i.e. vascular smooth muscle cells and pericytes, in arteries, veins and the microvasculature. In addition, the basic mechanisms of Ca^{2+} mobilisation, voltage-dependent and independent Ca^{2+} entry, internal store uptake and release of Ca^{2+} and Ca^{2+} extrusion/exchange pumps are coupled together in tissue-specific manners. It's the subtle variations in the combination of these mechanisms that are detailed in this book that establishes the unique functions of each smooth muscle organ.

This increase in complexity of cells present and their underlying mechanisms of rhythmicity reveals numerous new areas of investigation to accelerate our understanding of an individual smooth muscle's function and dysfunction, as well as to identify clinically relevant targets for pharmaceutical intervention. At present, there are numerous exciting gene-based techniques that are beginning to be applied to smooth muscle research, such as cell-specific expression of fluorescent marker proteins and the in situ visualisation and manipulation of cells, contraction or calcium using optogenetic techniques. Further transcriptome analyses of the molecular phenotype of the cells in particular smooth muscle organs will allow increasingly more cell-specific identification and mutation/knockin of channels or proteins using Cre-Lox genetic technology, even the time-determined in situ excitation/inhibition of particular cells with synthetic receptors that can be activated by unique ligands not found anywhere in the smooth muscle organ. All of these techniques will permit more sophisticated in situ investigations in animal models with engineered smooth muscle pathologies relevant to the human condition. This volume of reviews includes current knowledge obtained from the few early adopters of these genetic techniques based on their 'pre-genetic' investigations. It is hoped that the other reviews herein will also inform future research into the physiology and pathology of other spontaneously active smooth muscles.

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Introduction

The gastrointestinal (GI) tract displays various motility patterns, in the form of wiggling, expansion or constriction, and thus their contractile behaviour is in marked contrast with the heart which develops highly-coordinated, rhythmic beating. Textbooks of physiology describe the contractile activity of the GI tract as peristalsis that can be further divided into several patterns such as pendulous, propulsive or segmenting movements. These GI tract movements have been considered myogenic in origin, i.e., arising from the smooth muscle wall. Pacemaker cells that drive the spontaneous contractile activity appear to be distributed within the musculature, either scattered or forming a network, since similar contractile activity can be seen in different segments of the GI tract. The complex movements in segments of the GI tract are really mysterious and have attracted the interest of many smooth muscle researchers. Such spontaneous movements can also be seen in other smooth muscle tissues, such as those in the urinary tract, genital or vascular systems. However, the origin and cellular mechanisms, and sometimes even their precise physiological function, have yet to be elucidated.

Professor Edith Bülbring at University Oxford, one of the pioneers of smooth muscle research, was fascinated by the spontaneous rhythmic activity in smooth muscle tissues. Her achievements in understanding the mechanisms underlying spontaneous activity in smooth muscle tissues were remembered, together with the summary of the physiological and pharmacological properties of smooth muscles, in a book titled "*Smooth Muscle*", which was written together with her students, A.F. Brading, A.W. Jones and T. Tomita nearly 50 years ago [1]. Initially, the generation of spontaneous activity in smooth muscle tissues was considered to be tightly linked with cellular metabolism, since the activity was highly sensitive to the temperature. As lowering the temperature slows or prevents spontaneous movements, the metabolic changes in the smooth muscle cells were thought to modulate ionic mechanisms such as action potentials or slow potential changes (slow waves) that underlie muscle contractions [2]. The 'metabolic theory' was more clearly interpreted by Golenhofen [3] as follows; 'the metabolic changes occurred within smooth muscle cells (basic activity) elicit mechanical activity termed "minute rhythm" in smooth muscle tissues, by generating membrane electrical activity, and in physiological conditions the "minute rhythm" is further modulated by neuronal and humoral factors such as autonomic nerves or hormones'.

The electrical events generated in the cell membrane, in association with the spontaneous movement of smooth muscle tissues, are either in the form of action potentials or slow waves. They were considered to be myogenic in origin, since the spontaneous activity could be modulated but not prevented, by chemicals which block the actions of any neural influences. Putative pacemaker cells were thought to distribute within the smooth muscle tissues rather heterogeneously, since spontaneously electrical activity varied in their form and properties in a region dependent manner [4, 5]. Many attempts had been made to identify the localization of pacemaker cells. One of the most remarkable experiments was carried out in the isolated dog intestine, in which the pacemaker cells were predominantly distributed in the myenteric layers [6]. Thus, in the obliquely cut edge of the intestinal wall, the largest electrical activity of smooth muscle cells was recorded in the region between the circular and longitudinal smooth muscle layers.

The notion that specialised smooth muscle cells, i.e. pacemaker cells, are distributed within the musculature and generate spontaneous electrical activity that passively propagates to neighbouring smooth muscle cells to depolarize their membranes, is not astounding. In the heart, it was clearly demonstrated that pacemaker cells within the sino-atrial node generate pacemaker potentials that spread along specialized conducting muscle bundles to drive cardiac muscle cells in the cardiac chambers [7]. Thus, prior to the book "*Smooth Muscle*", it appears that studies of pacemaker mechanisms in smooth muscle were interpreted using the existing knowledge of cardiac pacemaking mechanisms, presumably due to the difficulty in the precise analysis of the electrophysiological properties of smooth muscle cells [1].

An important turning point in the research of smooth muscle rhythmicity is the discovery of the intestinal motility disorders observed in *c-kit* signaling deficient mice or *W/Wv* mutant mice [8]. Dr. H. Maeda (Kumamoto University) and his colleagues originally aimed to develop animal models that were devoid of mast cells to facilitate the understandings of their role in immune protective mechanisms. They generated *c-kit* deficient mice using *c-kit* antibodies since mast cells require the expression of *c-kit* gene encoding tyrosine kinase at the cell membrane for their growth and maturation. Thus, the occurrence of intestinal motility disorders in these *c-kit* deficient or mutant mice was a rather accidental and fortuitous finding. Professor K. Nishi at the Pharmacology Department of Kumamoto University immediately recognized the importance of Maeda's finding, and started to investigate what was happening in these *c-kit* deficient or mutant mice (K. Nishi, personal communication). Professors K.M. Sanders (University of Nevada at Reno) and J.D. Huizinga (McMaster University) further advanced the research on the mutant mice, and found that the diseased intestine had a lack of a population of cells called interstitial cells of Cajal (ICC) within the GI tract wall. Eventually, it has been established that these cells might be the pacemaker cells driving the spontaneous GI motility [9].

Ten years before the discovery of the *c-kit* deficient or mutant mouse, the fundamental roles of ICC in generating spontaneous activity of the GI smooth muscle had already been proposed by L. Thuneberg in 1982 [10], based on the distribution and morphological characteristics of these cells. He found

that interstitial cells distributed at the myenteric layer of intestine were rich in mitochondria and were connected with each other as well as neighbouring smooth muscle cells via gap junctions. It has already been known that the spontaneous activity of excised GI smooth muscle tissues is maintained for several hours if they are placed in a balanced oxygenated physiological salt solution kept at an adequate temperature. It was therefore reasonable to assume the importance of mitochondria as a source of energy supply in maintaining the spontaneous activity, while gap junctional connections would allow propagation of electrical activity generated in ICC to the smooth muscles cells. This unique proposal by the anatomist, however, was not recognized for a decade by the majority of smooth muscle researchers. Using methylene blue staining, the distribution of these mitochondria-rich cells in the gut wall was originally described by the Spanish anatomist S.R. Cajal, as being unidentified cells, either primitive nerves or interstitial cells (Historic background of ICC is reviewed in detail by Komuro et al. [11]).

The symposium on smooth muscle, organized by Professors T.B. Bolton (University College London) and T. Tomita (Nagoya University) took place in April 1995 at Nagoya, Japan, shortly after the joint meeting between the 72nd Physiological Society of Japan and Physiological Societies of Great Britain and Eire held at Nagoya University. These two organizers were well-known researchers of smooth muscle, and the presentations were summarized in a book "*Smooth Muscle Excitation*" [12]. The symposium had two stages, the 1st stage held in Nagoya for a couple of days was formal in style, while the 2nd stage was held on the Izu peninsula, again for two days, in a rather informal style, after an enjoyable bus trip. An important aspect of the symposium, especially in the 2nd stage, was the invitation of young students to the meeting. The idea for having the symposium in such a unique style, mixing young students and established researchers together was proposed by Professor T. Tomita. Smooth muscle research is often tough, largely due to the technical difficulties involved, so that the collection of data is excruciatingly slow. Thus, smooth muscle research has not been particularly attractive to young students. Professor T. Tomita aimed to encourage young students to understand that smooth muscle is not just a "*headache muscle*", rather it is a very interesting frontier.

In this symposium, Professors K.M. Sanders and J.D. Huizinga introduced the role of ICC in the spontaneous activity of the GI smooth muscle tissues [13, 14]. Although the role of ICC as the pacemaker of spontaneous activity of gut had already been published [9, 15], their presentations and notions were novel to many of the smooth muscle researchers present. In the poster presentation at Nagoya, the interpretation of the structural characteristics of ICC was described by Professor L. Thuneberg. It was the first time that I met him, and I found immediately that he was a really serious scholar who quietly presented his fundamental findings.

Nevertheless, at that time, I had some doubts as to the idea that ICC are the pacemaker cells in intestinal smooth muscle tissues. To that point, electrical activity had only been recorded in the smooth muscle cells of the intestine and there had been no demonstrations of propagation of electrical signals between ICC and the muscle layer. My doubts were clearly dissolved by the

elegant experiments carried out by Professor G.D.S. Hirst (Melbourne University), who successfully identified electrical activity in both ICC and smooth muscle cells using dye-injection during their recordings. The 'driving potential' (also known as pacemaker potential) generated in ICC had very steep upstroke followed by long plateau component, a time course completely different from the slow wave recorded in smooth muscle cells. Simultaneous recordings of the spontaneous electrical activity in both ICC and neighbouring smooth muscle cells indicated that these two types of cells were electrically coupled, and that the driving potential invariably preceded slow wave [16]. These results clearly showed that slow waves recorded from smooth muscle cells were formed upon electrotonic spread of depolarizing potentials generated in ICC.

There are several subtypes of ICC distributed in the GI wall, and some of them are directly innervated by myenteric or autonomic nerves [11, 17]. These histological and ultrastructural characteristics of ICC and their neighbouring smooth muscle cells may facilitate further interpretation of their function and how they influence the electrical and mechanical properties of the various regions of the GI tract [1].

A group of researchers, who were interested in the properties of ICC held the 1st International Symposium on Interstitial Cells of Cajal (ICC) in Lorne, Australia, in December 2002 (organized by G.D.S. Hirst). Although spontaneous movements had been known in many types of smooth muscle tissues, the symposium mainly focused on the ICC in GI tract, since the pacemaking role of ICC was recognized only in GI muscles at that time. There were 11 speakers in the symposium, and all the topics were related on the properties of GI smooth muscles. Since then, similar symposia had been held every year. The initial 2 symposia included papers related exclusively to ICC in GI muscles. However, later symposia contained papers relating to a variety of smooth muscle tissues. At the 5th International Symposium on ICC, held in August 2007 at County Monaghan, Ireland (organizer: Professor Noel McHale at the Dundalk Institute of Technology), the papers presented included topics on the urinary tract (renal pelvis, urinary bladder, urethra), arteries, corpus cavernosum, biliary tract and uterus, in addition to ICC in GI tissues. The 6th International Symposium on ICC, held in February 2010 at Miyazaki, Japan (organizer H. Suzuki) covered even wider topics relating to ICC, such as their histological and histochemical characteristics, as well as pacemaker cells different from ICC, neural regulation of the activities of pacemaker cells, intracellular mechanisms for generating automatic activity in pacemaker cells, and ion channels contributing the generation of pacemaker activity.

Thus, spontaneous activity is observed in many types of smooth muscle tissues, and this book covers the properties of smooth muscle cells in the GI tract, urinary tract (bladder, urethra, renal pelvis, ureter), reproductive system (oviduct, uterus, prostate, seminal vesicle, cavernosal tissue), airways as well as blood and lymphatic vessels. Intrinsic activity is produced by ICC distributed in smooth muscles of the GI tract, while 'similar-looking' interstitial cells distributed in smooth muscles of the uro-genital organs may have other roles. In GI smooth muscle tissues, ICC are histochemically characterised by their expression of c-kit protein at the membrane [11]. However, similar

expression occurs in other types of cells such as mast cells, so that the tandem expression of other markers such as vimentin and anoctamin-1 (Ano1 or TMEM 16A) have been developed to more specifically identify ICC [11]. Thus, the presence of cells immuno-positive to these markers has been used to suggest that these cells are pacemakers in a number of smooth muscle organs. However, these suggestions appear somewhat premature. For example, in the upper urinary tract c-kit negative interstitial cells display rhythmic activity which modulates pacemaker activity generated by atypical smooth muscle cells [18] (Chap. 3). Uterine smooth muscle tissue is rather unique, although this tissue has a distribution of c-kit positive interstitial cells that are not rhythmically active. Their role has yet to be determined, but it has been suggested that they act as transducers of electrical signals between different regions of smooth muscle cells [19] and that some of the smooth muscles themselves act as the organ's pacemaker (Chap. 10). Interestingly, the involvement of ICC in the sphincter muscles of the GI tract, and their important role in maintaining the sustained contractions of these smooth muscles have been established (Chap. 2). Sustained contraction of smooth muscle cells is also known in the corpus cavernosum to maintain the penis in an flaccid condition, but in this case with there no involvement of ICC-like cells (Chap. 7). Thus, there seem to be a large divergence in the role of ICC or ICC-like cells in smooth muscle tissues, again highlighting that tissue-specificity is a significant feature of smooth muscle organs and that the results obtained in intestinal muscles are not always applicable to other smooth muscle tissues. These variations might be one of the characteristic features of smooth muscle tissue so that detailed analysis of individual tissues is required before a comprehensive understanding of their pacemaker mechanisms is obtained.

Associated with the confirmation of the histological properties of pacemaker cells in individual smooth muscle tissues, the cellular mechanism of the generation of pacemaker potential has also been given attention. Again, an understanding of the mechanisms in GI ICC is further advanced compared to pacemaker mechanisms in other types of smooth muscle tissue. Tokutomi et al. [20] first showed the possible involvement of Ca^{2+}-sensitive Cl^- current in the generation of pacemaker potentials in intestinal ICC. However, the contribution of Cl^--current was not initially accepted, because it was rather difficult to explain the data obtained in experiments using large tissue segments. The spontaneous electrical activity of smooth muscle in the guinea-pig stomach, for example, remained unaltered, or only slightly reduced, in solutions containing lowered Cl^- concentrations [4, 5]. In hindsight, it is now understood that the interpretation of experimental results using solutions with modified ionic compositions is difficult, due to the involvement of unexpected non-specific actions of the substitutions on the smooth muscle cells and/or ICC.

It is generally known that simplification of systems will facilitate the understanding of their mechanisms and experiments using single cells, or sometimes a piece of cell membrane, have been used extensively in the last 30 years, to study pacemaking mechanisms in smooth muscle and their pacemaker cells. Techniques developed also include the measurement of Ca^{2+}

dynamics in cells and their intracellular compartments such as sarcoplasmic reticulum or mitochondria, the activity of single ion channels distributed in the membranes of cell wall or cell organelles, structural modulation of ion channels by changing the sequence of amino acids of channel proteins, double staining of targeted cells with several protein specific antibodies, and more recently the transgenic modulation or identification of particular cell types. The analysis of the function of pacemaker cells using many of these "new" techniques will be found in chapters included in this book.

In isolated gastric muscle tissues of the guinea-pig, the co-ordination between an elevation of intracellular Ca^{2+} concentration in ICC and the generation of a pacemaker potential in ICC was clearly shown by Hirst et al. [21]. Possible involvement of inositol trisphosphate (IP_3) in the initial steps of the generation of pacemaker potential was also suggested in the stomach, where gastric muscles isolated from IP_3-receptor knock-out mice did not display slow waves [22]. It was speculated that the periodical production of IP_3 triggers the release of tiny packets of Ca^{2+} from the internal stores, and this local elevation of intracellular Ca^{2+} activates Cl^- channels to elicit a transient depolarization of the membrane, called unitary potentials [16], or spontaneous transient depolarizations (STDs) as observed in lymphatic tissues [23]. When the amplitude of summed STDs or unitary potentials exceeds the threshold level for the activation of T-type Ca^{2+} channels, a driving (or pacemaker) potential is generated in ICC. These processes were proposed over 15 years ago [24], and although additional data may be required for a fuller interpretation of the ICC pacemaker mechanisms of ICC, this initial observation still seems to be relevant in the understanding of spontaneous electrical activity in intestinal smooth muscle tissues. Of course, now the mechanisms of the spontaneous movements of smooth muscle tissues is being examined in more detail at sub-cellular levels which are also described in some chapters in this book. The authors of each chapter of this book are all well-established researchers in their field and leading experts in the function of the smooth muscle tissue/organs they study.

Our understandings on the cellular mechanism of spontaneous movements in smooth muscle tissues has thus greatly improved since the discovery of the pivotal role of ICC as the pacemaker cells in the GI tract. Readers of this book, however, will also find that ICC have multiple roles, such as pathways of low resistance conduction between ICC themselves and neighbouring smooth muscle cells, intermediaries of neural signals from myenteric or autonomic nerves to smooth muscle cells, or acting as mechanical sensors of stretch in the GI wall. In other smooth muscle tissues such as urogenital organs, lymphatic vessels or reproductive organs, it is less likely that groups of cells having histological or histochemical similarities to ICC are undertaking a pacemaker role driving spontaneous contractile activity. Moreover, it remains to be determined whether these cells have roles other than pacemaking, as do ICC in the GI tract. Many smooth muscle tissues distributed in urogenital organs are known to be sensitive to factors such as hormones, while their intrinsic activity also changes depending on their physiological conditions. One typical examples can be found in uterine smooth muscle, where the spontaneous irregular activity of the smooth muscle wall in

non-pregnant rats is changed to a periodical generation of burst of spikes during pregnancy [25]. Most smooth muscle tissues also change their spontaneous activity in response to the hormonal and humoral conditions, and it remains unclear whether these changes include their pacemaker cells directly or indirectly through the change in the properties of their smooth muscle cells. Thus, there are a lot of questions to be solved, and I presume many of them will be clarified soon. I am confident that the contents of this book will greatly advance the understanding of smooth muscle function and encourage further analysis of the remaining mysteries underlying smooth muscle rhythmicity.

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Part I

Gastrointestinal Tract

1 Spontaneous Electrical Activity and Rhythmicity in Gastrointestinal Smooth Muscles

Kenton M. Sanders

Abstract

The gastrointestinal (GI) tract has multifold tasks of ingesting, processing, and assimilating nutrients and disposing of wastes at appropriate times. These tasks are facilitated by several stereotypical motor patterns that build upon the intrinsic rhythmicity of the smooth muscles that generate phasic contractions in many regions of the gut. Phasic contractions result from a cyclical depolarization/repolarization cycle, known as electrical slow waves, which result from intrinsic pacemaker activity. Interstitial cells of Cajal (ICC) are electrically coupled to smooth muscle cells (SMCs) and generate and propagate pacemaker activity and slow waves. The mechanism of slow waves is dependent upon specialized conductances expressed by pacemaker ICC. The primary conductances responsible for slow waves in mice are Ano1, Ca^{2+}-activated Cl^- channels (CaCCs), and $Ca_V3.2$, T-type, voltage-dependent Ca^{2+} channels. Release of Ca^{2+} from intracellular stores in ICC appears to be the initiator of pacemaker depolarizations, activation of T-type current provides voltage-dependent Ca^{2+} entry into ICC, as slow waves propagate through ICC networks, and Ca^{2+}-induced Ca^{2+} release and activation of Ano1 in ICC amplifies slow wave depolarizations. Slow waves conduct to coupled SMCs, and depolarization elicited by these events enhances the open-probability of L-type voltage-dependent Ca^{2+} channels, promotes Ca^{2+} entry, and initiates contraction. Phasic contractions timed by the occurrence of slow waves provide the basis for motility patterns such as gastric peristalsis and segmentation. This chapter discusses the properties of ICC and proposed mechanism of electrical rhythmicity in GI muscles.

Keywords

Interstitial cells of Cajal · Pacemaker · Ca^{2+} transient · Slow wave · SIP syncytium · ANO1 channels · T-type Ca^{2+} channels · Electrophysiology · Gastrointestinal motility

1.1 Introduction

GI smooth muscles are complex tissues composed of multiple cell types. Smooth muscle cells (SMCs) provide the motor responsible for force development and movement of nutrients and waste products, but at least two additional types of cells, known as interstitial cells, are electrically coupled to SMCs and provide moment-to-moment modulation of SMC excitability.

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Together with SMCs, interstitial cells of Cajal (ICC) and platelet-derived growth factor receptor α positive (PDGFRα$^+$) cells form a multicellular syncytium known as the SMC, ICC, and PDGFRα$^+$ cell (SIP) syncytium [1]. SIP cells each express intrinsic electrophysiological mechanisms and a variety of receptors for neurotransmitters, hormones, paracrine substances, and inflammatory mediators. SIP cells are innervated by enteric motor neurons and receive and transduce neurotransmitter signals. The integrated output of the SIP syncytium sets the moment-to-moment excitability of SMCs [2]. The contractile behavior in most smooth muscle regions of the GI tract is phasic in nature, consisting of a rhythmic contraction-relaxation cycle. Phasic contractions are the basis for the major motility patterns of the GI tract, such as segmentation and peristalsis, and the phasic nature of contractions is intrinsic to the muscle tissues. Phasic contractions of smooth muscle cells (SMCs) are driven by electrical slow waves, which are generated by ICC [3–5]. There are several types of ICC present in the GI tracts of mammals and humans. Common nomenclature used to identify the different classes of ICC and categorize their functions is provided in Table 1.1.

ICC are organized into networks in the pacemaker regions of the GI tract. Once a slow wave is generated, it regenerates cell to cell, propagating actively through the ICC network. Slow waves conduct passively into SMCs, because SMCs do not express the unique conductances that contribute to slow waves and therefore have no means for their regeneration (i.e., depolarization of a SMC does not produce a slow wave-like event). Depolarization of SMCs by slow waves enhances the open-probability of L-type voltage-

Table 1.1 Nomenclature for ICC in the GI tract

Anatomical location	Common name	Organ distribution	Functions
Plane of the myenteric plexus between circular and longitudinal muscle layers	ICC-MY[a]	STM, SI, CLN	Pacemaker activity, innervated by motor neurons in CLN
Intramuscular localization, within muscle bundles and in close contact with varicose processes of enteric motor neurons	ICC-IM[b]	ESG (smooth muscle portion), STM, SI, CLN	Express receptors for and provide transduction for neurotransmitters released by enteric motor neurons; mediators of responses to stretch
Intramuscular-type ICC within plane of the deep muscular plexus in small intestine	ICC-DMP[c]	SI	Express receptors for and provide transduction for neurotransmitters released by enteric motor neurons
Submucosal border of circular muscle layer	ICC-SM	CLN, STM	Pacemaker activity in CLN; limited number of cells in STM and function of STM cells unknown
Serosal surface of longitudinal muscle layer	ICC-SS	CLN	Unknown function at present time
Septal spaces between muscle bundles in larger animals	ICC-SEP	STM, SI, CLN	Appear to be extensions of ICC-MY or ICC-SM networks and actively propagate slow waves in thicker GI muscles of large mammals and humans

Organ abbreviations: Esophagus (ESG); stomach (STM); small intestine (SI); colon (CLN)

[a]Also referred to as ICC-MP by some authors, but this is misleading because these cells do not penetrate and are not part of the myenteric plexus. They are distributed around the ganglia and tertiary plexus

[b]Some authors have broken this term down to specify in which muscle layer the cells are found (e.g., ICC-CM for cells in the circular muscle layer and ICC-LM for cells in the longitudinal muscle layer). Since no functional differences have been reported for the cells in these different locations, the term ICC-IM is used throughout this chapter

[c]ICC-DMP are most likely the ICC-IM of the small intestine. They show a distinctive localization in laboratory animals and have received considerable experimental attention, so they are designated separately. Larger animals tend to have ICC-IM distributed through the circular muscle layer, as observed in the stomach and colon of laboratory animals.

dependent Ca^{2+} channels that are ubiquitously expressed in GI SMCs. In some SMCs of the small bowel and colon activation of L-type Ca^{2+} channels results in generation of Ca^{2+} action potentials, which are superimposed upon the peaks of slow waves. In the stomach slow waves depolarize SMCs but action potentials are not typically generated. In either case Ca^{2+} entry into SMCs initiates contraction (excitation-contraction coupling), and in both cases the slow wave depolarization/repolarization cycle determines the period of enhanced open probability of L-type Ca^{2+} channels in SMCs (contraction) and the period of time in which Ca^{2+} channel open probability is low (relaxation). This chapter discusses the characteristics of slow waves in GI muscles, the apparatus required for slow waves, the mechanism of slow wave generation and propagation, and how nerves and other factors influence cells of the SIP syncytium to increase or decrease the gain for excitation-contraction coupling. It should also be noted that in some regions of the GI tract, such as the internal anal sphincter [6], slow waves occur at sufficient frequencies to cause summation of cytoplasmic Ca^{2+} and tonic contraction (similar to a partial tetanus), but this topic is covered in another chapter.

1.2 Nature of Electrical Rhythmicity in GI Smooth Muscles

Contractile rhythmicity in GI organs was likely recognized as soon as the abdomens of freshly killed animals were sliced open. Gut motility persists for various periods of time after the death of an animal because it is not driven by circulating factors in blood or by neural input from the central or enteric nervous systems. The basic motility patterns are intrinsic to the cells and tissues of the *tunica muscularis*, and cells of the SIP syncytium appear to be rather resistant to the hypoxia that rapidly kills the heart and brain. Placing metal electrodes on organs of the gut allowed electrical recording of gut activity nearly 100 years ago [7, 8], but it is likely that these recordings were heavily contaminated by movements. At the time of the initial recordings the electrophysiological basis for muscle contraction was unknown, and it was not possible to block movements independently of upstream mechanisms. Therefore, we have no way of knowing whether the first electrical recordings of GI muscle activity contained electrophysiological information (i.e., events based on changes in transmembrane potentials in cells within the muscles or organs) or were "biopotentials" resulting from muscle movements [9]. Better techniques developed with time, such as sucrose gap, which was a means of obtaining pseudo-transmembrane potential recording, provided valuable information about the waveforms of electrical slow waves in the gut [10, 11]. Voltage-clamping of GI muscle strips was attempted with sucrose gaps, and several ideas about the mechanism of slow waves were developed based on these experiments [12, 13]. However, true voltage-control (and space clamp) of the many electrically coupled cells in the SIP syncytium may have been difficult to accomplish with this approach.

Eventually cell impalement techniques were adopted to directly measure transmembrane potential in a dynamic manner [14–17]. Small SMCs are difficult to impale, and impalements of cells among moving muscle cells are difficult to maintain, but this technique provided, and still provides, the most accurate means of measuring resting membrane potentials, slow waves, and action potentials in intact muscles (Fig. 1.1). Microelectrode studies allowed investigators to better understand the ionic mechanisms that cause slow waves, the responses to neurotransmitters, and the effects of bioactive compounds. Information obtained from these recordings is dependent upon many voltage- and non-voltage-dependent and receptor-operated ion channels expressed in cells of the SIP syncytium [18]. It remains technically difficult to voltage-clamp intact GI muscles, except perhaps with small bundles of cells, as used by David Hirst and collaborators in many studies of smooth muscle tissues [19–21]. It should be reemphasized that GI muscles are syncytial in nature, and the complete syncytium (SIP syncytium) contains SMCs, ICC, and PDGFRα^{+} cells [1]. Thus, intracellular record-

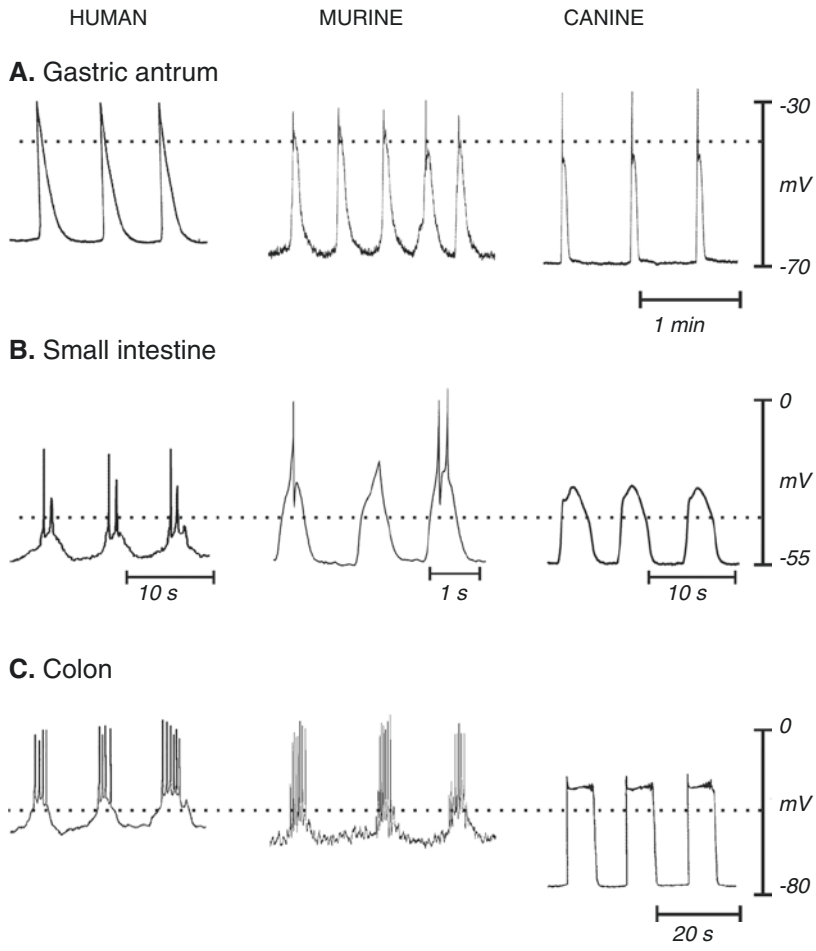

Fig. 1.1 Electrical activity recorded from stomach, small bowel, and colon of three species. Recordings were made with intracellular microelectrodes from the circular muscle layers of isolated strips of muscle from the antrum, ileum or jejunum and proximal colon. The major features of electrical activity that vary in waveform in different regions of the GI tract and in different species are displayed. From a relatively stable membrane potential between slow waves (resting membrane potential), a sharp upstroke depolarization occurs when a propagating slow wave reaches the point of recording. The upstroke typically repolarizes quickly to a pseudo-stable plateau potential that can last for several seconds before repolarization to the resting potential. Resting potentials vary, making it necessary for slow waves in different regions to depend upon different voltage-dependent Ca^{2+} channels to carry the main current during the upstroke (see text for details). The plateau potential depends upon sustained activation of Ano1 channels that are activated by Ca^{2+} release events in the ER of ICC. In some regions slow waves initiate Ca^{2+} action potentials in SMCs. These are initiated in the small bowel and colon when the depolarization reaches about −40 mV (dotted lines in each panel). Ca^{2+} action potentials are superimposed upon the slow wave plateau phase. Slow waves with or without superimposed action potentials generate phasic contractions. Copied with permission from [2]

ing from a single cell within the SIP syncytium is complex and contains membrane potential information not only from the impaled cell but also from electrically coupled cells. The complexity of the SIP syncytium was unknown to investigators during the early use of both sucrose gap and intracellular microelectrode recording, and several behaviors believed to be intrinsic to SMCs are now known to originate in cells other than SMCs (e.g., slow waves, fast (purinergic) inhibitory junction potentials, cholinergic excitatory junction potentials (EJPs); see [4, 5, 22, 23]).