Are interstitial cells of Cajal plurifunction cells in the gut?

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Are interstitial cells of Cajal plurifunction cells in the gut? Am J Physiol Gastrointest Liver Physiol 294: G372–G390, 2008. First published October 11, 2007; doi:10.1152/ajpgi.00344.2007.—The proposed functions of the interstitial cells of Cajal (ICC) are to 1) pace the slow waves and regulate their propagation, 2) mediate enteric neuronal signals to smooth muscle cells, and 3) act as mechanosensors. In addition, impairments of ICC have been implicated in diverse motility disorders. This review critically examines the available evidence for these roles and offers alternate explanations. This review suggests the following: 1) The ICC may not pace the slow waves or help in their propagation. Instead, they may help in maintaining the gradient of resting membrane potential (RMP) through the thickness of the circular muscle layer, which stabilizes the slow waves and enhances their propagation. The impairment of ICC destabilizes the slow waves, resulting in attenuation of their amplitude and impaired propagation. 2) The one-way communication between the enteric neuronal varicosities and the smooth muscle cells occurs by volume transmission, rather than by wired transmission via the ICC. 3) There are fundamental limitations for the ICC to act as mechanosensors. 4) The ICC impair in numerous motility disorders. However, a cause-and-effect relationship between ICC impairment and motility dysfunction is not established. The ICC impair readily and transform to other cell types in response to alterations in their microenvironment, which have limited effects on motility function. Concurrent investigations of the alterations in slow-wave characteristics, excitation-contraction and excitation-inhibition couplings in smooth muscle cells, neurotransmitter synthesis and release in enteric neurons, and the impairment of the ICC are required to understand the etiologies of clinical motility disorders.

smooth muscle; slow waves; enteric neurons; excitation-contraction coupling; peristaltic reflex; motility disorders; volume transmission; synaptic transmission, ICC

The interstitial cells were characterized originally by Raymond V. Cajal (20, 21). These cells got his attention and that of others because they are intercalated between the autonomic nerves and the effector cells. On the basis of the understanding of the interneuronal communication in the central nervous system (CNS) at that time, Cajal proposed that the intercalating cells [later called the interstitial cells of Cajal (ICC)] mediate the transmission of signals from the autonomic neurons to the smooth muscle cells. A few years later, Keith (84) observed a collar of similar cells in the myenteric plexus of the rat ileocecal junction. He was looking for a pacemaker of the gut smooth muscle electrical activity because a few years earlier he had identified such a pacemaker in the sinoatrial node of the heart. He hypothesized that those interstitial cells were the pacemaker cells in the gut. In the late 1960s and early 1970s, several investigators described a plexus of the ICC within the myenteric and submucosal plexi of the small and large intestines. They also identified close connections between the enteric axonal varicosities and ICC processes on one side and gap junctions between the ICC processes and the circular smooth muscle cells on the other side (51, 59, 115, 155). Daniel, with a strong background in smooth muscle cells, viewed the intercalation of ICC between the enteric neuronal varicosities and smooth muscle cells from another viewpoint. He hypothesized that the ICC act as mechanoreceptors in the gut wall.

Thus over a span of about 70 years, ICC were proposed to regulate three primary gut motility functions. These proposals were based almost entirely on circumstantial ultrastructural observations by light and electron microscopy. All three proposals were apparently logical, but they lacked functional and mechanistic evidence. Needless to say, our understanding of the smooth muscle and enteric neuronal electrophysiology, excitation-contraction coupling in smooth muscle cells, and the chemical coding of the enteric neurons was meager at that time, compared with what it is now.

In a landmark paper in 1982, Thuneberg (168) focused the interest in gastrointestinal ICC by providing an elegant and thorough summary of the work accomplished on the ICC until that time. More importantly, he integrated the histological and light microscopic findings of other investigators with those of his own and consolidated several hypotheses that became the subjects of intense research over the next 25 years. His findings indicated the following: 1) There are four loci of ICC in the mouse small intestine. He classified them as ICC-I, ICC-II, ICC-III, and ICC-IV. This review will use a more contempo-
Regulation of Gut Motility Function by ICC

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Invited Review

Pertinent Background on Topics Essential to Evaluate the Roles of ICC in the Regulation of Gastrointestinal Motility Function

The mutant mouse (W/Wv, sl/sld) and rat (Ws/Ws) models for investigation of the roles of ICC in gut motility function. Three mutant models, W/Wv and sl/sld mice and Ws/Ws rat, have been used to investigate the roles of the ICC in the regulation of gut motility function. The mouse models have mutations at the spotting W and steel loci, which code the c-kit receptor tyrosine kinase and the kit ligand (also called steel factor), respectively (14, 23, 103). The c-kit receptor tyrosine kinase belongs to the PDGF/CSF-1/c-kit receptor subfamily. Both mutations cause deficiencies in gametogenesis, melanogenesis, and hematopoiesis (165, 171).

Selective subtypes of the ICC are obliterated, reduced in numbers, or damaged at different locations in the gastrointestinal tract of these mutant animals. However, these models of developmental mutations are complex because they produce multiple defects, including sterility, coat color abnormalities, severe macrocytic anemia, and mast cell deficiency. These defects can indirectly produce developmental deficiencies in other cells that do not express c-kit by changing their microenvironment during or after embryonic development.

As an example, the spontaneous or synthetic mutations of the endothelin-3 (edn3) or endothelin receptor B (ednrb) have been used to suppress the development of the enteric nervous system (ENS) in the colon of lethalspot(lsl s) mice. Homozygous mutations of the genes that encode the receptor tyrosine kinase, Ret, or its ligand, glial cell-derived neurotrophic factor (gdnf), produce aganglioneosis in mice (52, 126). The mutation of ednrb or edn3, which encodes a ligand to ednrb, also causes distal intestinal aganglioneosis in rodents and humans (75). Although the target of these mutations are the precursor cells of the enteric neurons, these models also exhibit abnormalities of structure and function in the surrounding cells, such as the smooth muscle cells (166). Slow waves are absent in the circular smooth muscle cells of the aganglionic segment in patients with Hirschsprung’s disease (89) and in mutant rodent models of aganglioneosis, even though the enteric neurons are not involved in the generation of slow waves in wild-type animals (25, 90). The cytoskeleton proteins are also markedly reduced in the smooth muscle cells of the aganglionic bowel in Hirschsprung’s disease (109). The contractility of smooth muscle cell in response to ACh is increased in the lsl s mice, indicating potential alterations of excitation-contraction coupling in smooth muscle cells (69).

It is noteworthy that, although c-kit is deemed essential for the development and the maintenance of the ICC, the c-kit or steel gene mutation does not uniformly obliterate different ICC subtypes, or even the same ICC subtype in different organs of the gastrointestinal tract. The gastric ICC-MY have been reported variously as not affected (48) or reduced in numbers in discrete patches in different parts of the stomach (117). By contrast, the ICC-MY in the small intestine are obliterated almost completely (80, 183), whereas those in the colon are severely reduced in numbers (1). The ICC-IM are obliterated in the stomach but not in the small intestine. The reasons for the differential effects of the same mutations on the same subtypes of the ICC in different gut organs are not fully understood. Although some structural differences among the different subtypes of the ICC have been noted, it is not known whether the subtypes of the ICC have distinct phenotypes. It is not known also whether the same subtype of the ICC in different organs have the same phenotype. For example, what makes the gastric ICC-MY to generate slow waves, whereas the gastric ICC-IM generate regenerative potentials and mediate neuronal input? Furthermore, some subtypes of ICC cannot be identified by c-kit immunoreactivity (180). Do these cells have a different phenotype than those that are immunoreactive to c-kit? It seems that the dependence of different subtypes of ICC on c-kit in a gut organ as well as that of the same subtype of ICC in different gut organs is variable.
The relative roles of slow waves, excitation-contraction coupling, excitation-inhibition coupling, and enteric motor neurons in the regulation of rhythmic phasic contractions in the gut. Two of the proposed roles of the ICC are to generate and propagate slow waves and to mediate the enteric neural input to the smooth muscle cells. Ultimately, the smooth muscle cells in the gut wall generate the force of contraction that causes the motility functions of mixing and propulsion. This section provides a brief background to the interactions among the mechanisms that regulate the motility function by generating three different types of gut contractions. The readers are referred to other reviews for an in-depth treatment of these interactions (94, 106, 133, 140).

Smooth muscle contraction occurs by interaction between actin and myosin filaments by cross-bridge cycling (106, 140, 152). The amplitude of the force generated and the duration of cell contraction depend on the intensity and duration of cross-bridge cycling, which, in turn, depends on the intensity and duration of phosphorylation of the 20-kDa myosin light chain (MLC20). MLC20 is phosphorylated by myosin light chain kinase (MLCK) and dephosphorylated by myosin light chain phosphatase (MLCP) (Fig. 1). The phosphorylation of MLC20 is regulated by two mechanisms 1) electromechanical coupling and 2) pharmacomechanical coupling. The binding of the cytosolic-free calcium to calmodulin activates MLCK. MLC20 is phosphorylated mildly in the resting state of the cell by low concentration of cytosolic-free calcium (70–100 nM), which maintains the resting tone.

The slow wave is periodic depolarization of smooth muscle membrane that is generated by ionic mechanisms independent of voltage-dependent Ca,1.2 (L-type) Ca\textsuperscript{2+} channels. The RMP of the gut smooth muscle cells, recorded in an organ bath, varies roughly in the range of −50 to −70 mV. The maximum membrane potential achieved during a slow-wave depolarization varies from species to species and from organ to organ, but it is generally in the neighborhood of about −45 to −40 mV. The membrane depolarization during a slow wave induces calcium influx via the Ca,1.2 channels (129). The current-voltage curves indicate very small inward current during the maximum membrane depolarization of the slow waves to about −40 mV (Fig. 2A) (101). As a result, during a slow wave depolarization, the smooth muscle cell contracts with a tiny force by the phosphorylation of MLC20 by MLCK, as described (Figs. 1 and 2B). The contraction that is solely due to the membrane depolarization by slow wave is called electromechanical coupling. Although these tiny contractions are recordable in in vitro preparations, they do not effectively constrict the lumen in the intact state, and, therefore, they have little or no effect on motility function (77). A good example of these tiny contractions is the quiescent state in the stomach and the small intestine during phase I of the migrating motor complex (MMC) cycle. The slow waves continue during this phase, but there are no spikes (action potentials) superimposed on each slow wave and there is little or no contractile activity. Correspondingly, there is no propulsion of a dye bolus during phase I of the MMC cycle (157).

The excitation of cholinergic motor neurons during a slow-wave depolarization releases ACh. The binding of ACh to the M3 receptors on smooth muscle membrane triggers pharmacomechanical coupling, which significantly enhances the amplitude of contractions by several mechanisms (Fig. 1). I) The opening of receptor-operated Ca\textsuperscript{2+} channels (RGCC) by excitation-contraction coupling further increases Ca\textsuperscript{2+} influx, which depolarizes the membrane beyond the mechanical excitation threshold, also known as spike-generation threshold (Fig. 2B) (8). The membrane depolarization beyond the mechanical excitation threshold induces a burst of spikes that are electrical oscillations at 5–10 Hz. The membrane depolarization during each spike exceeds 0 mV into the range where inward calcium current is maximal (Fig. 2A) (101). Together,
all the spikes in a burst cause massive influx of calcium. This calcium influx dramatically increases the cytosolic calcium concentration ([Ca\(^{2+}\)]), the activation of MLCK, phosphorylation of MLC20, and contractile force. The amplitude of a rhythmic phasic contraction (RPC) in the intact state relates to the number of spikes superimposed on the slow wave, rather than to the amplitude of the slow wave (45, 66).

2) The activation of the M3 muscarinic receptors stimulates several signaling pathways that reduce the activity of MLCP, which increases the rate, amplitude, and duration of phosphorylation of MLC20, and it further enhances the amplitude and duration of the contraction. This is called the adjustment of Ca\(^{2+}\) sensitivity (152).

3) The generation of inositol 1,4,5-triphosphate (IP3) by the excitation-contraction coupling also releases Ca\(^{2+}\) from the endoplasmic stores, reducing Ca\(^{2+}\) influx, and by increasing the activation of MLCP (decreasing Ca\(^{2+}\) sensitivity). The reader is referred to other elegant reviews for further discussion of cell signaling for excitation-contraction and excitation-inhibition couplings in gut smooth muscle cells (106).

The above background has relevance to an important putative role of the ICC, i.e., to mediate the enteric neuronal input to the smooth muscle cells. If this is, indeed, one of the roles of the ICC, they must regenerate ACh in response to its release from the excitatory motor neurons. Only the direct binding of ACh on smooth muscle muscarinic receptors activates the excitation-contraction coupling in smooth muscle cells (Fig. 1). Specifically, an electrical signal transmitted from the ICC to the smooth muscle cells cannot activate the excitation-contraction coupling, and hence it would not stimulate effective smooth muscle contractions.

It is noteworthy also that the overall gut motility function is regulated by the generation of the following three distinct types of contractions: 1) RPCs, 2) ultrapropulsive contractions...
(UPCs), which are of two types, giant migrating contractions (GMCs) and retrograde giant contractions (RGCs), and 3) tone (133, 140). Table 1 summarizes the specific contributions of slow waves, excitation-contraction coupling, and enteric neurons to the generation and propagation of the three types of gut contractions. The slow waves make major contributions only to the regulation of RPCs (Table 1). This review focuses primarily on the slow waves and the RPCs.

The modes of intercellular communication. The intercellular communication in the CNS and the periphery occurs by two means.

Wired transmission. Wired transmission is fast one-to-one transmission. It includes communication by classic synapses, gap junctions, and membrane juxtapositions (Fig. 3A). The wired transmission is discrete and localized, where high concentrations of the mediators (ions, classic neurotransmitters, and neuropeptides) are achieved in the closed synaptic cleft (100 micromolar to millimolar range), and they act on low-affinity postsynaptic receptors. The neutralization of high concentrations of the neurotransmitter in the synaptic cleft is by their uptake (e.g., GABA, glutamate, and lysine) or enzymatic degradation (ACh). The frequency of the incoming pulses in the neurons encodes the transmission signal. The source to target distances in the wired transmission is in the range of 2–50 nm, and the source to target delay is in the range of microseconds to milliseconds. This type of transmission is essential for fast transmission of specific audio, video, and stored memory signals from the source to target areas.

The wired transmission also occurs when informational molecules directly pass from the source to target cells, such as that in gap junctions (electrical synapses). The spatial buffering of K⁺ and Ca²⁺ in the gap junctions can affect the excitability of target cells, such as that among smooth muscle cells in the periphery and in neuronal-glial networks in the CNS and the periphery (15, 175). It is noteworthy that such intercellular electrical interactions also can occur at the plasmic membrane juxtapositions of the excitable neuronal and nonneuronal cells by intercellular exchange of ions. The effectiveness of interaction reduces, and the time delay increases with greater distances at these juxtapositions.

Volume transmission. Volume transmission is slower, one-to-many and many-to-one transmission (68, 88, 111, 160, 176, 178, 179, 201) (Fig. 3B). It includes paracrine and endocrine-like transmission in the extracellular space and in the cerebrospinal fluid. The volume transmission is diffuse and relatively slow (milliseconds to minutes). The transmitter concentrations are low in this type of transmission due to its mixing with the extracellular fluid. The transmitter may diffuse also from leaky synapses or from nonjunctional varicosities where high densities of small vesicles exist in varicosities lacking synaptic specializations. These varicosities are located far away from any recognizable postsynaptic targets. This is classified as a mismatch between the release site and the target receptor in morphological studies (47, 83, 116).

Most studies that have concluded that the ICC act as mediators of the enteric neuronal input to the smooth muscle cells have tacitly assumed that the intercellular communication in the gut occurs only through synapses and gap junctions. This is simply not true. Both modes of intercellular communication are utilized in the CNS and the periphery. The utilization of one or both modes depends on the specific needs of the organ.

The Roles of ICC in the Generation and Propagation of Slow Waves

Which cells generate spontaneous slow waves? The finding that most subtypes of the ICC express c-kit and the availability of mice and rats with c-kit or steel mutations (WW and sltsk mice and Ws/Ws rats) made it possible to investigate the potential role of the ICC in the generation of the slow waves in the smooth muscle cells. The “pacemaker hypothesis” is that the ICC-MY in the stomach and the small intestine and the ICC-SM in the colon generate slow waves, which then propagate electrotonically (passively) into the thickness of their respective circular muscle layers via gap junctions. Because of the passive cable-like conduction, the amplitude of the slow waves decreases with distance from the source of their generation. However, a closer examination of the available evidence, along with the information presented in Pertinent Background on Topics Essential to Evaluate the Roles of ICC in the Regulation of Gut Motility Function, indicates alternate explanations of the source of generation of slow waves in the gut.

Despite the selective and patchy lesioning (alterations of morphology, density, and gradient of expression between the corpus and the antrum) of the gastric ICC-MY (117), no differences in the characteristics of the slow waves are found between the stomachs of the intact awake WW and the wild-type mice (76). The findings are divergent in the organ bath. One report found that the slow waves are absent in a significant number of the circular smooth muscle cells in the WW mice, but they occur in all cells in the wild-type mice (117). Another report found that the slow waves are present in all antral cells in the WW mice, but they are attenuated in amplitude and lack an inflection (secondary component or the regenerative potential) near the peak of the slow wave (48). Yet another report found that the gastric slow waves in an organ bath in the WW mice are not different from those in the wild-type mice (18). These conflicting findings highlight the

Table 1. Contributions of core regulating mechanisms to the spatiotemporal characteristics of different types of gut contractions

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complexities of the mutant models. The slow waves in both the W/W<sup>+</sup> and the wild-type mice are insensitive to nifedipine, indicating that similar ionic mechanisms underlie their spontaneous generation.

To investigate the role of the ICC-MY in the generation of gastric slow waves, strips of gastric muscle tissues were incubated with the neutralizing antibody to Kit (ACK2) for 31–50 days. This obliterated all the ICC, including the ICC-MY, which are resistant to c-kit mutation, and abolished the slow waves in the circular muscle cells (119). However, the incubation with ACK2 also depolarizes the smooth muscle cells to about −40 mV, which is greater than the reversal potential of gastric slow waves (36, 113, 119). The smooth muscle cells cease to generate slow waves when their membrane is depolarized beyond the slow-wave reversal potential. Therefore, the abolition of the slow waves in these experiments is due to the depolarization of the circular smooth muscle cells beyond the slow-wave reversal potential, rather than due to the absence of the pacemaker activity of the ICC-MY.

The ICC-MY are almost totally absent in the small intestine of the adult W/W<sup>+</sup> and sl/sld mice and Ws/Ws rats (165, 183, 184). Some organ bath studies reported almost complete absence of the slow waves in these mice (Fig. 4, A and B) (183, 184). Others found that the small intestine of the intact W/W<sup>+</sup> mice and muscle strips in organ bath continue to generate slow waves and RPCs, but both are more irregular in frequency and smaller in amplitude than those in the wild-type mice (Figs. 4, C–F) (45, 76, 77, 80, 105). The slow waves and their associated contractions in the W/W<sup>+</sup> mice propagate over shorter distances than those in the wild-type mice. The divergent findings in the same animal model point to differences in the interpretation of data. The continuation of the rhythmic activity in the complete absence of the ICC-MY in the small intestine was puzzling and contrary to the pacemaker hypothesis. It was
explained by assuming that the membrane potential oscillations in \( \text{W}^+ \text{W}^- \) mice were not slow waves, but they were “slow-wave action potentials,” which have the rhythmicity of the slow waves (Fig. 4D) (45, 107). However, there is no explanation as to what initiates the periodic occurrence of discrete spike bursts and their associated phasic contractions in these mice. According to the information in Pertinent Background on Topics Essential to Evaluate the Roles of ICC in the Regulation of Gut Motility Function, the membrane potential must depolarize briefly beyond the mechanical excitation threshold for the gut smooth muscle cells to generate spikes and contract for a brief period to stimulate phasic contractions (Fig. 2B). An obvious alternate explanation is that the circular smooth muscle cells generate slow waves, but the slow waves are destabilized (smaller amplitude and phase unlocked) in the absence of the small intestinal ICC-MY in the \( \text{W}^+ \text{W}^- \) mice (Fig. 4, D and F).

Similarly, in the colon of the Ws/Ws rats, in which the presence of all subtypes of ICC is severely compromised, the slow waves as well as the RPCs regulated by them continue, albeit with less regularity and at smaller amplitudes (1, 64, 197). The physical separation of the myenteric plexus in the stomach and the small intestine, or that of the submucosal plexus in the colon, abolishes or disorganizes the slow waves in the remaining circular smooth muscle cells (8, 36, 96, 124, 143, 151, 159). Taken together, the findings in these studies and those in the mutant animals suggest that the potential role of the ICC-MY in the stomach and the small intestine of ICC-SM in the colon may be to stabilize the slow waves generated spontaneously in the circular muscle cells, rather than to pace them. The obvious question, therefore, is what factor or factors in these plexi regulate the stability of slow waves generated autonomously by the circular smooth muscle cells. The answer to this question lies in the following heterogeneous electrophysiological characteristics of the circular smooth muscle cells through the thickness of the circular muscle layer (2, 3, 8, 123, 143, 151). 1) The circular smooth muscle cells exhibit a gradient in their RMP from the myenteric plexus to the submucosal plexus in the stomach and the small intestine and from the submucosal plexus to the myenteric plexus in the colon (Fig. 5A) (8, 98, 150, 151). The slope of the RMP gradient is greater in the colon than in the stomach and the small intestine (162). The amplitude of the slow wave and the generation of spikes are highly dependent on the resting membrane (1, 8, 74, 105, 150, 151). Consequently, the characteristics of the slow waves and their ability to generate spikes are heterogeneous through the thickness of the circular muscle layer. 2) The amplitude of the slow wave decreases progressively with depolarization of the RMP (Fig. 5A) (2, 123, 151, 158). Note that the shape and configuration of the slow waves also depend on the amplitude of the RMP. 3) Regardless of what the RMP is, the maximum membrane potential reached during a slow-wave depolarization remains about the same, which is close to but slightly less than the mechanical (spike) excitation threshold (8, 119, 123, 158). This means that if the RMP becomes very close to the mechanical excitation threshold, the slow wave amplitude may become too small to be detected visually (Fig. 5A). This mechanical threshold is different in different organs of the gut, but usually it is in the range of about \(-45\) to \(-35\) mV. The slow waves may cease to exist if the RMP exceeds the slow-wave reversal potential (8, 49, 158). The slow-wave reversal potential is nearly the same or slightly more depolarized than the mechanical excitation threshold. The significance of the above heterogeneous electrophysiological characteristics is that the smaller amplitude slow waves in the depolarized circular muscle cells have nearly the same ability to reach or exceed the mechanical excitation threshold and stimulate phasic contractions, as that of the larger amplitude slow waves generated in hyperpolarized smooth muscle cells. Both the small and the large amplitude slow waves require the presence of ACh to depolarize to a potential greater than the mechanical excitation threshold (Fig. 2C).

The RMP gradient is impaired almost consistently in all studies that have used the mutant animals, separated one of the plexi from the circular muscle layer, or incubated naïve tissues with ACK2 or methylene blue (8, 34, 36, 45, 74, 100, 105, 130, 141, 143, 183). The resulting RMP is depolarized closer to that of the smooth muscle cells near the myenteric plexus in the colon and the submucosal plexus in the stomach and the small intestine. This indicates that the myenteric plexi in the stomach and the small intestine and the submucosal plexus in the colon contain a factor or factors that hyperpolarize the circular smooth muscle cells closer to them. The influence of this factor decreases with distance away from the respective plexi. The decrease in the RMP away from these plexi results in a corresponding gradual decrease in slow-wave amplitude (Fig. 5).

The smooth muscle cells generating slow waves in the gastrointestinal tract behave as arrays of relaxation oscillators (138, 139). In a system of coupled relaxation oscillators, each...
cell generates autonomous slow waves, whose characteristics depend solely on the conditions within the cell, such as the RMP. The slow waves in the adjacent cells influence each other’s frequency and onset of depolarization by electrical coupling between them at the intercellular junctions. The intensity of coupling between two adjacent cells depends upon the amplitude of oscillation in each cell and the coupling factor between them. The morphology of the junction, including the intercellular distance, determines the coupling factor. The RMP gradient in the thickness of the circular muscle layer and the relaxation oscillator characteristics of the smooth muscle cells explain the following features of the slow waves in the wild-type and mutant animals. 1) The onset of the slow waves at different locations in the thickness of the intact circular muscle strips is almost simultaneous, but their amplitudes decrease from the submucosal plexus to the myenteric plexus in the colon and from the myenteric plexus to the submucosal plexus in the stomach and the small intestine (Fig. 5, A and B) (151). The slow waves are synchronous because all the circular smooth muscle cells throughout the thickness of the layer oscillate at nearly the same frequency, and they are tightly coupled. The amplitude of the slow wave decreases away from the submucosal plexus in the colon and away from the myenteric plexus in the stomach and the small intestine because the RMPs of the cells decrease in these directions. This decrease in slow-wave amplitude is not due to its decay by passive conduction, such as that which occurs in a cable. The reported length and time constants do not explain the nearly simultaneous onset of slow waves through the thickness of the circular muscle layers (Fig. 5B) (72, 79, 82). 2) The reduction in the amplitude of the slow waves in W/W<sup>−</sup> mice is due to the reduction or the elimination of the RMP gradient rather than due to the absence of the pacemaker activity of the ICC-SM in the colon and ICC-MY in the stomach and the small intestine. 3) The presence of the slow waves may not be detected visually if the RMP of the circular muscle cells after elimination of the RPM gradient approaches close to the slow-wave reversal potential (45, 80, 183). However, fast Fourier transform analysis of the electrical activity clearly detects the slow-wave oscillations (1, 76). The power of this signal is reduced due to the smaller amplitude of the slow waves, resulting from the depolarization of RPM. 4) The decrease in the propagation of slow waves and their associated RPCs in the mutant animals is due to impaired coupling among the neighboring circular smooth muscle cells. This impairment of the coupling is due to the reduction in the slow-wave amplitude.

The lack of consistent generation of slow waves by dissociated circular smooth muscle cells cannot be taken as evidence that they do not generate them spontaneously and consistently in the intact state. It seems that, compared with the ICC, the smooth muscle cells are more labile in generating slow waves in isolation. There is evidence, however, that the circular smooth muscle cells generate spontaneous slow waves, spikes, and their associated phasic contractions when the dissociated cells are clumped together (54). A percentage of single dissociated smooth muscle cells also generates spontaneous slow waves (123). Furthermore, a significant percent of the colonic circular muscle strips separated from the ICC-SM generate spontaneous slow waves and RPCs. Those circular smooth muscle strips that do not generate slow waves spontaneously in isolation can be stimulated pharmacologically to generate them (96, 97). This demonstrates that the circular smooth muscle cells have the necessary ionic mechanisms to generate slow waves autonomously, but these mechanisms may be impaired by alterations in their microenvironment (33, 96).

Other factors may also contribute to the suppression of slow-wave activity in single dissociated smooth muscle cells. As noted above, the circular smooth muscle cells depolarize, when separated from the myenteric plexus in the stomach and the small intestine and the submucosal plexus in the colon. If the depolarization exceeds the slow-wave reversal potential, the generation of slow waves may cease. Furthermore, the enteric neurotransmitters, such as ACh and VIP, have dual roles. They induce the excitation-contraction and excitation-inhibition couplings (Fig. 1), and they induce excitation-transcription coupling in smooth muscle cells (144, 145). This excitation-transcription coupling maintains the expression of specific key cellular signaling proteins, such as the ion channel and the contractile proteins. In the dissociated state, the cells are no longer exposed to these enteric neurotransmitters. The consequent alterations in the expression of some of the cell signaling proteins may further depolarize the membrane potential or alter their characteristics so that the isolated circular smooth muscle cells cease to generate spontaneous slow-wave oscillations. For example, the K<sup>+</sup> channels play a key role in maintaining the RMP of the gut smooth muscle cells. The alterations in calcium release by IP<sub>3</sub> receptors also lead to suppression or loss of slow-wave activity (16, 42, 99, 158). The only sure way to determine that the circular smooth muscle cells do not generate spontaneous slow waves is to record from them with microelectrodes in intact W/W<sup>−</sup> or Sl/Sl mice or the Ws/Ws rats. Such recordings have been made in intact small intestine of anesthetized dogs (38).

On the other hand, there is little doubt that the ICC generate robust slow waves of large amplitude in the intact state, and they also generate them in isolation (93). The quandary, however, is that the networks of the ICC-MY and ICC-SM form tightly coupled uniform two-dimensional syncytia via gap junctions along the circumference and the length of the organs. Therefore, the frequency of the slow waves generated over substantial areas of these networks would be phase locked because of the strong coupling among the ICC. If the ICC acted as the pacemakers, the frequency of the slow waves generated in the underlying circular smooth muscle cells would also be the same and phase locked along substantial lengths of each of the major organs in the gut. However, the slow waves in the colon and the distal small intestine are variable in frequency and not phase locked (132, 134, 135, 138). The answer to this quandary is unknown. Further investigations are needed to determine how the slow waves generated independently in the ICC-MY and in circular smooth muscle cells interact to influence the regulation of RPCs in the gut. It is noteworthy that the tight coupling between the ICC and smooth muscle cells, which is an important basis of the pacemaker hypothesis, has been questioned (37, 40, 43).

The role of the gap junctions, one of the critical components of the pacemaker hypothesis, has been questioned (12, 16, 40, 142). The disruption of the gap junctions by pharmacological agents has little effect on the slow-wave activity in the circular muscle cells of intact muscle strips (40, 43, 142). Other studies have demonstrated poor coupling between the gastric ICC-MY and the adjacent circular smooth muscle cells (37, 41). Note
that the longitudinal muscle cells of the guinea pig colon generate spontaneous contractions regulated by the slow waves even after their separation from the myenteric plexus and ICC-MY (153). The autonomous pacemaker activity is present throughout the thickness of the circular muscle layer in the canine gastric antrum, not just in the isolated myenteric plexus (74). These findings suggest that the circular smooth muscle cells generate the slow waves autonomously, but their characteristics, such as the amplitude and wave shape, change when the ICC are impaired.

Which factor or factors in the myenteric plexus of the stomach and the small intestine and the submucosal plexus in the colon maintain the RMP gradient through the thickness of the intact circular muscle layer? The next obvious question is which factor or factors in the enteric plexi maintain the gradients of the RMP through the thickness of the circular muscle layer. It is noteworthy that the ICC or any other excitable cell could not conduct the RMP gradient electrotonically to its neighboring excitable cells because the steady potentials, like the RMP, do not conduct across capacitative junctions. Therefore, the larger RMP in the ICC-MY or the ICC-SM could not be the factor that maintains the gradient of RMP in the circular muscle layer. This mediator may be a readily diffusible molecule that hyperpolarizes the smooth muscle cells. Two such potential molecules are NO and carbon monoxide (CO). An interesting series of recent studies suggests that CO generated by the ICC-MY in the stomach and the small intestine and by the ICC-SM in the colon may produce the observed gradients of the RMP in their respective circular muscle layers (55, 62, 162, 193). Heme oxygenase (HO) activity and CO production correlate well with the observed gradients of the RMP through the thickness of the circular muscle layers in the stomach, small intestine, and the colon (55, 104). These gradients are absent in HO-2 knockout mice (193). The ICC-MY in the stomach and small intestine and the ICC-SM in the murine colon are immunoreactive to HO-2. It remains to be investigated whether other factors in these plexi also contribute to the maintenance of the RMP gradient.

NO is also a gaseous molecule that hyperpolarizes smooth muscle cells. Even though the enteric neurons are immunoreactive to HO-2, they may not be the source of CO to maintain the gradient of the RMP. Tetrodotoxin does not abolish the RMP gradient. In addition, this gradient is present in c-RET-null mice that lack the ENS (120). The smooth muscle cells are also not the source of CO because they are not immunoreactive to HO-2 (55).

Note that the RMP gradient also regulates the amplitude and stability of the slow waves along the lengths of the organs. The RMP is smaller (more depolarized) in the fundus, and it increases distally toward the antrum (55, 161). Correspondingly, the amplitudes of the slow waves increase from the corpus to the antrum. In the fundus, the smooth muscle membrane is depolarized greater than the slow-wave reversal potential so that the fundic smooth muscle cells do not generate spontaneous slow waves. Similarly, the RMP is greater (more hyperpolarized) in the duodenum than in the ileum (104). Accordingly, the amplitude and the stability of the slow waves decrease from the duodenum to the ileum (138). CO generated from the breakdown of HO also correlates with the RMP gradients from the gastric fundus to the gastric antrum and from the duodenum to the ileum (55, 104).

Which cells regulate the propagation of slow waves? The ICC-MY in the stomach and the small intestine, and the ICC-SM in the colon are arranged in uniform two-dimensional syncytium via gap junctions (131). These ICC networks have been proposed to regulate the propagation of slow waves in the longitudinal and circumferential axes of the gut. If so, the propagation of the slow waves should occur at identical speeds along the length and the circumference of these organs. However, the propagation of slow waves is much faster along the circumference than along the length of the gut organs (72, 139), which does not fit with their propagation in the ICC plexi. The differential velocities of propagation of the slow waves along the two axes allow the circular smooth muscle cells along the circumference to contract nearly simultaneously, resulting in a ring-like contraction at a given location. At the same time, the slower and variable rates of propagation of slow waves along the long axis allow slow and variable propagation rates of the RPCs along the length of the organs. Furthermore, in areas of the gut, such as the ileum and the colon, the RPCs propagate very little to produce much slower transit times, compared with those in the stomach and the proximal small intestine. However, these regions have the syncytium of the ICC-MY and ICC-SM, which are very similar to those in the proximal gut. By contrast, the intercellular distances between the circular smooth muscle cells vary along the length of the gut, and they are different between the longitudinal and circumferential directions. This structural organization is consistent with different propagation velocities and distances of propagation of slow waves and the RPCs in different directions and in different organs.

The propagation of the slow waves in the colon is impaired by the separation of the submucosal plexus (50, 53). This impairment is likely due to the destabilization of the slow waves by elimination of the RMP gradient, rather than due to the absence of the ICC-SM syncytium. The depolarization of the circular muscle cells on their separation from the submucosal plexus reduces the amplitude of the slow waves. As discussed in an earlier section, this reduces the coupling between neighboring cells and causes instability of slow waves.

In summary, therefore, the ICC and the circular smooth muscle cells may generate slow waves independently. The slow waves generated autonomously in the smooth muscle cells do not necessarily require pacing from those generated in the ICC. In fact, the pacing of circular smooth muscle slow waves by the ICC slow waves can create logistical problems because of the presence of a two-dimensional syncytium in the ICC but not in the circular smooth muscle cells. This pacing is not consistent with the observed rapid propagation of slow waves along the circumference but slow and variable propagation along the long axis of the gut organs. A potential role of specific subtypes of the ICC may be to generate CO that diffuses through the thickness of the circular muscle layer to maintain a gradient of the RMP. This gradient enhances the amplitude of the slow waves in the circular muscle cells closer to the ICC, which stabilizes the slow waves across the thickness of the circular muscle layer. This gradient also improves the effective coupling between the smooth muscle cells so that the slow waves and, hence, the contractions regulated by them can propagate.
One way to test the “RMP gradient hypothesis” is to determine whether the slow waves are destabilized in the HO-2-null mice that have normal expression of ICC but no gradient of the RMP. However, other factors in the mutant models, such as alterations in the expression of ion channels, specifically the K+ channels, which make a significant contribution to the RMP, and of other cell signaling proteins that may alter the calcium handling by circular smooth muscle cells may also contribute to the destabilization of slow waves in the mutant models.

Does Communication Between the Enteric Neurons and Smooth Muscle Cells in the Gut Occur Indirectly by Wired Transmission or Directly by Volume Transmission?

This section discusses the feasibility of the wired and volume transmissions for communication between the enteric axonal varicosities and gut smooth muscle cells. In the synaptic transmission (wired transmission) model, the excitatory and inhibitory neurotransmitters are released in the synapses between the axonal varicosities and the target ICC. The ICC transduce the chemical signal into an electrical signal and transmit it electrically to the smooth muscle cells with which they make gap junctions. Note that the total volume of all subtypes of the ICC in the gut is about 5–7% of the total volume of the circular smooth muscle cells. Therefore, in the model of synaptic transmission, the ICC can deliver the electrical signal directly only to a fraction of all the smooth muscle cells. In the volume transmission model, the excitatory and inhibitory neurotransmitters released by the axonal varicosities of the enteric motor neurons diffuse in the extracellular fluid to the surface of the adjacent smooth muscle cells to stimulate their respective target receptors.

Wired (synaptic and gap) transmission. Morphological and ultrastructural studies show that the processes of specific subtypes of ICC form synapses with nerve varicosities on one hand and gap junctions with a fraction of the circular smooth muscle cells on the other hand (10, 12, 20, 39, 73, 180, 188). The molecular, immunohistochemical, and ultrastructural approaches have identified the molecular identities of the pre- and postsynaptic proteins [synaptotagmin (SNAP-25) and postsynaptic densification (PSD-93) and (PSD-95)], which are similar to those of the synapses in the CNS and the skeletal neuromuscular junction. There is, therefore, little doubt that fast, one-to-one chemical communication occurs between the enteric neurons and the classes of ICC proposed to act as mediators of neuronal input. The ICC also can communicate effectively with the smooth muscle cells with which they form gap junctions.

Numerous studies have attempted to support the notion of synaptic and gap-junction transmission between the enteric neurons and smooth muscle cells by examining the generation of postjunction potentials in circular smooth muscle cells of the mutant animals, which lack specific subtypes of ICC.

Stomach. The ICC-IM that are thought to mediate the postjunction potentials in circular smooth muscle cells of the stomach are attenuated in the gastric fundus and the antrum of these mice compared with those in the wild-type mice. The remaining component of the IJPs in W/Wv mice is sensitive to apamin, which suggests that unlike NO and ACh, ATP may diffuse directly from the axonal varicosities to the smooth muscle cells. The density and the distribution of the NADPH-IR neurons are similar, and the smooth muscle cells relax normally to VIP in both the mutant and the wild-type mice. However, besides obliterating the gastric ICC-IM, c-kit mutation also alters the enteric neuronal and smooth muscle functions. The NO donor, sodium nitroprusside, produces membrane hyperpolarization and concurrent relaxation of smooth muscle tone in muscle strips from the wild-type mice. In the mutant mice, it fails to induce membrane hyperpolarization in spite of relaxation of the smooth muscle tone. The release of ACh by EFS is reduced in the W/Wv mice. The nature of the smooth muscle contractile response to neostigmine is also altered in the mutant mice (57). Therefore, the causes of the attenuated amplitudes of IJPs and EJPs in c-kit and steel factor mutations may be more complex than simply due to the ablation of specific subtypes of ICC, as discussed in the previous sections.

Small intestine. In the small intestine, the ICC-IM that mediate the muscular plexus (DMP), but not the ICC-IM, are intercalated between the axonal varicosities and circular smooth muscle cells (86, 128, 180, 181, 200). On the basis of this morphological observation, the ICC-DMP are thought to mediate the enteric neuronal input to the circular smooth muscle cells in this organ. However, the motor neurons from the myenteric plexus project directly into the circular muscle layer (17, 60, 110, 112, 170). Indirectly, the above hypothesis implies the lack of a role of the motor neurons in the myenteric plexus in regulating the small intestinal motility function. This scenario is highly unlikely. The possibility that the myenteric motor neurons synapse on the neurons in the deep muscular plexus and so they indirectly regulate the motility function is also unlikely and unproven. The acceptance of the “ICC-DMP hypothesis” leaves a major conflict in our understanding of the myenteric neural regulation of intestinal motility function. It is also noteworthy that the ICC-DMP exhibit very little immunoreactivity for neuronal NO synthase (nNOS). Therefore, it is not clear how they would transmit the signal from the inhibitory motor neurons to the smooth muscle cells with which they make gap junctions.

The ICC-DMP are not immunoreactive to c-kit, and they are not obliterated in W/Wv mice. Therefore, there are no hard data to prove the hypothesis that they mediate the enteric neuronal input to the inner circular smooth muscle cells. Two indirect approaches have been used. The neonatal mice exhibit postjunction responses on day P10 but not on day P0. It was hypothesized that the late appearance of these responses to EFS is due to the late development of the ICC-DMP. However, the incomplete functional development of the enteric neurons on day P0 cannot be ruled out. This possibility cannot be tested by immunostaining alone. In other experiments, the incubation of naïve P0 tissues with c-kit antibody for 10 days obliterated the ICC and reduced the postjunction responses when compared with those in tissues incubated with the culture medium alone. However, the toxic effects of incubation with ACK2 on the function of the enteric neurons and smooth muscle cells cannot be ruled out.

Colon. The amplitude of the IJP in the canine colonic circular smooth muscle layer is not altered when it is detached from the submucosal and the myenteric plexi containing the...
ICC-SM and the ICC-MY, respectively (78). A recent study in 
Ws/Ws rats also shows that severe depletion or the absence of 
all subtypes of colonic ICC has no significant effect on the IJPs 
in the circular smooth muscle cells (1). The ascending excitato-
dory and descending inhibitory reflexes induced by balloon 
distension of colon segments from the W/W mice are also not 
altered when compared with those in the wild-type mice (114). 
It seems that the inhibitory motor neurons innervate the ICC-SM 
and the circular muscle cells in parallel (78). More importantly, 
the postjunctional potentials due to the direct inhibitory motor 
inervation of the circular muscle cells do not depend on the 
parallel innervation of the ICC-SM or that of the ICC-MY.

The ICC in most species are only marginally immunoreac-
tive to nNOS. However, the canine colonic ICC-SM and 
ICC-MY express nNOS and VIP receptors, but they do not 
express VIP immunoreactivity (13, 78, 174, 192). Furthermore, 
the enteric axonal varicosities that make close contacts with 
ICC-IM contain NOS-like immunoreactivity and vesicular 
ACh transporter-like immunoreactivity. In acting as interme-
diaries for nitrergic neurotransmission between the enteric 
neurons and smooth muscle cells, the ICC amplify the NO 
signal (125). The exogenous application of NO on colonic ICC 
increases their intracellular Ca\(^{2+}\) to activate NOS. The NO, 
thus generated, further increases the generation of NO by 
positive feedback. Thus the output of NO from the ICC in 
response to a brief stimulus can last for several minutes. The 
NO generated in an ICC can diffuse to the nearby smooth 
muscle cells up to a minimum distance of 225 \(\mu m\) to decrease 
their intracellular calcium concentration ([Ca\(^{2+}\)])). However, 
an increase of [Ca\(^{2+}\); in smooth muscle cells does not affect 
their neighboring smooth muscle cells. Therefore, the NO 
generated in the ICC would affect only the smooth muscle cells 
close to them, and this effect does not propagate in smooth 
muscle cells. These findings show a unique ability of the 
colonie ICC-SM to amplify the neuronal NO signal; they also 
show that NO can diffuse effectively to distances far greater 
than those between nerve varicosities and smooth muscle cells. 
Thus the NO released from nerve varicosities could directly 
affect smooth muscle cells by diffusion in the intercellular 
medium (volume transmission).

SPHINCTERS: The spatial distribution of the ICC-IM and their 
anatomical relationships with the enteric neurons and smooth 
muscle cells in the lower esophageal sphincter (LES) and 
internal anal sphincter (IAS) are similar to those in the rest of 
the gut organs (44, 149, 167, 186). The ICC-IM are almost 
totally absent in the sphincters of the W/W mice.

LES. Intact nitrergic innervation in the LES is critical for its 
relaxation in response to swallows (24, 121, 194). One would 
expect, therefore, that if the ICC-IM mediated the nitrergic 
inhibitory input to the smooth muscle cells, the LES relaxation 
would be impaired in the intact W/W mice. However, the 
percent LES relaxation in response to a swallow or vagal 
stimulation is not significantly different between the W/W and 
the wild-type mice (149). The attenuation of LES relaxation by 
nitro-L-arginine methyl ester is similar in both the wild-type 
and the W/W mice. By contrast, the swallow-induced LES 
relaxation is significantly impaired in the nNOS\(^{-/-}\) mice. 
These in vivo data indicate that the ICC-IM may not mediate 
the inhibitory input to the smooth muscle cells to relax the LES 
tone, even though they are strategically positioned between 
the enteric neurons and smooth muscle cells.

The LES pressure is largely due to myogenic tone. The LES 
pressure is lower in the W/W mice than that in the wild-type 
mice. The attenuation of the LES smooth muscle tone in the 
W/W mice may be secondary to c-kit mutation during develop-
ment. The hypotensive LES in the W/W mice also argues 
against the loss of nitrergic input to the smooth muscle cells in 
the W/W mice due to the absence of the ICC-IM because the 
loss of nitrergic input in nNOS\(^{-/-}\) mice enhances the resting 
LES pressure (149).

In contrast to the above findings in intact animals, the 
amplitude of the IJP is attenuated in the LES muscle strips 
from in the W/W mice when compared with that from the 
wild-type mice (186). However, in vitro studies also show 
that the NO donor, sodium nitroprusside, fails altogether to 
relax the muscle strips from the W/W mutant mice. These and 
other similar observations highlight the need to thoroughly 
examine the secondary alterations of the cell signaling proteins 
in the smooth muscle cells and the enteric neurons in the gene 
mutation models for accurate interpretation of data. Morpho-
logical and immunohistochemical examinations alone may not 
detect these subtle alterations.

INTERNAL ANAL SPHINCTER. The in vivo relaxation of the IAS 
response to rectal balloon distension in the W/W mice is not 
different from that in the intact wild-type mice (167). Another 
study found, however, that the in vivo relaxation is signifi-
cantly attenuated in the W/W mice (44). However, both studies 
found that the relaxation of the internal anal muscle strips in 
response to EFS is not affected in the W/W mice when 
compared with that in the wild-type mice. The in vivo discrep-
ancy between the two studies is, therefore, unlikely to be due 
to the lack of mediation of the nitrergic input to the smooth 
muscle cells by the ICC-IM in the W/W mice. The in vivo 
as well as in vitro relaxations are absent in the nNOS\(^{-/-}\) mice. 
Consequently, in this organ also there is little evidence that the 
ICC mediate the enteric neuronal input to the smooth muscle 
cells. Interestingly, the resting tone of the IAS is also lower in 
the W/W mice than that in the wild-type mice. The resting 
tone in this sphincter is regulated also by the myogenic mech-
anism as well as epithelial NOS (167). The alterations in the 
myogenic regulatory mechanisms, secondary to c-kit mu-
tation, may contribute to the reduction in the basal tone.

Overall, there are several fundamental problems with ICC 
acting as the mediators of the enteric neuronal input to the 
smooth muscle cells. 1) As indicated in the Pertinent Back-
ground on Topics Essential to Evaluate the Roles of ICC in the 
Regulation of Gut Motility Function, ACh must bind directly 
to the muscarinic receptors on the smooth muscle membrane 
stimulate excitation-contraction coupling. This means that if 
the ICC mediated the excitatory input, they must generate ACh 
of their own and release it at their gap junctions with the 
circular smooth muscle cells to elicit effective contrac-
tions. The ICC are nonneural cells, and they do not synthesize 
ACh. 2) Because of the small volume of ICC-IM (~6%) (180) 
compared with that of the circular muscle cells, the ICC do not 
make gap junctions with all the circular smooth muscle cells. 
Therefore, even if they could synthesize ACh, they would not 
be able to supply it to the entire population of smooth muscle 
cells. If so, not all circular smooth muscle cells will initiate 
excitation-contraction coupling to contract the gut wall effec-
tively. 3) The excitatory and the inhibitory motor neurons 
release their respective neurotransmitters concurrently. It is not
known how the cell signaling stimulated by each neurotransmitter in the ICC would interfere with each other and how the net resultant signal would be transmitted to the smooth muscle cells with which they make gap junctions. This net signal is unlikely to be an electrical potential. The electrical signals transmitted from the ICC cannot stimulate excitation-contraction and excitation-inhibition couplings in smooth muscle cells. There appears to be little advantage in adding an extra layer of ICC to repeat the neuronal function, particularly, when the total volume of the ICC is a small fraction of the total volume of smooth muscle cells.

In summary, there is little doubt that specific subtypes of ICC are strategically intercalated between the enteric axonal varicosities and a fraction of the total mass of the circular smooth muscle cells. This anatomical relationship favors a one-to-one and fast communication between these ICC and the enteric neurons on one hand and the ICC and the smooth muscle cells on the other hand. The ICC also express many of the receptors that the smooth muscle cells and the enteric neurons express. Thus they are in a position to recognize the release of some of the enteric neurotransmitters or gaseous molecules from the enteric neurons. However, this does not insure that the ICC can or may transduce the input from the enteric neurons into a signal to the smooth muscle cells that regulates a known gastrointestinal motility function. The overall in vitro data for a potential role of ICC as obligatory mediators is conflicting in different organs, but there is compelling in vivo data that is indicative of a lack of this role of the ICC.

**Volume transmission.** The axonal and dendritic branches emanating from a single neuronal cell body distribute randomly in three dimensions in the circular muscle layer, and there are \(10–20\) varicosities per \(100\) smooth muscle cells \((39, 58, 112)\). With volume transmission, the neurotransmitter released from a single varicosity reaches several smooth muscle cells in its neighborhood (Fig. 6). Furthermore, a single smooth muscle cell receives the neurotransmitter from several varicosities of different neurons surrounding it (Fig. 6). Consequently, the dilution of the neurotransmitter, as it diffuses through the intercellular medium as well as its reuptake or degradation, is not an issue. This arrangement insures a nearly uniform distribution and concentration of the neurotransmitter around the circumference of a short \(1–2\)-cm-long segment of the gut. It positions all the cells around the circumference in a short segment to contribute to the overall strength of the ring-like contraction, which is essential for effective mixing and propulsive functions of gut motility. Most importantly, in this arrangement, the neurotransmitters can bind directly to their receptors on each smooth muscle cell to stimulate their respective excitation-contraction, excitation-inhibition, and excitation-transcription couplings, which are essential to achieve effective strengths of gut contractions. The volume transmission also maintains an ambient level of the neurotransmitters to generate the basal tone as well as to generate tonic contractions and tonic inhibition of contractions. An ambient level of ACh is maintained by volume transmission in the CNS \((46, 47)\).

The original hypothesis for one-way communication between the enteric neurons and smooth muscle cells was based on volume transmission \((19)\). The experimental and functional data in the ENS and the CNS show that the effective distances for volume transmission are dramatically greater than the distances between the varicosities and neighboring smooth muscle cells in the gut wall \((125, 201)\). Furthermore, the receptors outside of the synapses have several-fold greater affinity for their ligands than those within the synapses \((177)\). It is noteworthy that the high affinity receptors outside of the synaptic junctions mediate most effects of therapeutic drugs. These receptors are much more readily accessible to them than those inside the shielded synapses and gap junctions are. The administration of high doses of therapeutic agents to access the shielded low affinity receptors may be toxic to other cells.

A recurrent theme in most studies that have proposed ICC as obligatory mediators of enteric neuronal input to the smooth muscle cells is that they form gap junctions of distances less than \(10\) or \(20\) nm with the smooth muscle cells and synaptic junctions of distances less than a few nanometers with the

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**Fig. 6.** In volume transmission, each circular smooth muscle cell concurrently may receive the neurotransmitter from several varicosities \((A)\), and each varicosity may concurrently supply the neurotransmitter to several smooth muscle cells \((B)\).
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enteric neuronal varicosities. The implication is that intercellular communication in the gut can occur only across these gap junctions and synapses. There is no scientific basis to adopt this distance as the cutoff point for intercellular communication in the gut or in other organs. Only a few studies have objectively investigated the intercellular distances between different cell types in the gut wall (39, 149). The “Match and Mismatch” paradigm used in the CNS would provide a clearer and objective picture of the players in intercellular communication in the gut (68). Volume transmission is better suited for one-way enteric neuronal-smooth muscle communication in the gut because 1) all the circular muscle cells along the circumference of a short segment must contract concurrently, 2) the desired communication speeds are slow, and 3) the number of smooth muscle cells is several-fold greater than that of the axonal varicosities, which necessitates one-to-many type of communication.

**ICC as Mechanosensors**

Most of the present data to support a role of the ICC acting as mechanoreceptors in the gut are on the basis of morphological studies (168, 169). The physiological data regarding a potential role of the ICC as mechanoreceptors is available only from the mouse gastric antrum (190). The underlying hypothesis is that the ingestion of a meal stretches the antrum. The antral ICC-IM sense this stretch. The sensing mechanisms in the ICC-IM transiently depolarize the smooth muscle cells with which they make contacts to increase the slow-wave frequency and decrease its amplitude by about 30–40%. The basis for this hypothesis is that the antral circular muscle strips from the W/W<sup>v</sup> mice, in which the ICC-IM are absent, do not exhibit the transient depolarization or the increase in slow-wave frequency and decrease in its amplitude. The lack of this response in the mutant mice may be due, in part, to the increase in the intrinsic slow-wave frequency of the antral circular smooth muscle cells (117). The proposed physiological significance of the ICC-IM acting as mechanoreceptors may be that after a meal, differential stretches occur in the corpus and the antrum, which uncouple their slow waves to modulate the rate of gastric emptying. Alternately, the stretch generated by the occurrence of a contraction at a given location may generate this sensory signal. If this hypothesis is correct, the postprandial slow waves should be slower in frequency and better propagating in the absence of the ICC-IM in the W/W<sup>v</sup> mice than in the wild-type mice. However, these data are not available in intact awake W/W<sup>v</sup> mice. The data on the alterations in the gastric emptying rates in the W/W<sup>v</sup> mice and how much of that can be attributed to the absence of the sensory function of the ICC-IM also are also not available. The characteristics of the gastric slow waves in the fasting state in W/W<sup>v</sup> mice, however, are not different from those in the wild-type mice (76).

There are fundamental difficulties with the basic concept of ICC-IM acting as mechanoreceptors in the gut wall. 1) Evidence is lacking that the antral wall is stretched or that there is a substantial increase in the antral slow-wave frequency after a meal. Other studies show that no differential intraluminal pressures are generated in different parts of the stomach after a meal, which does not support the differential stretches between the antrum and the corpus (95). The absence of the disruptive mechanosensory signal in the W/W<sup>v</sup> mice suggests that the postprandial slow waves, and hence the RPCs, will not be disrupted. The uninterrupted propagation of postprandial RPCs should accelerate gastric emptying (65), but there is no evidence to show that the gastric emptying is faster in the W/W<sup>v</sup> mice than that in the wild-type mice. 2) The ICC-MY in the stomach have been proposed as pacemakers of the slow waves in the circular smooth muscle cells as well as the synchronizers of the regenerative potentials in the ICC-IM. It is not apparent how the signal generated by the mechanical stretch in the ICC-IM, can increase the pacemaker frequency of the ICC-MY, which would, in turn, increase the frequency of the slow waves in the circular muscle cells (11, 71). One report suggests that on cholinergic stimulation, the ICC-IM become the dominant pacemakers in the antrum, but the molecular mechanisms of this switch are not known (70). 3) ICC-IM cannot produce membrane depolarization in smooth muscle cells with which they make gap junctions. Steady-state or slowly varying potentials do not conduct well across gap junctions, which are capacitative in nature. 4) Two functions were proposed previously for the gastric ICC-IM, one to generate the regenerative potentials, and the other to mediate the enteric neuronal input to the smooth muscle cells (18, 182). The mechanosensory function of these cells is the third. Each one of these functions requires intracellular signaling. Some of the intracellular signaling molecules or ion channels will be utilized by more than one function. It may not be feasible for the ICC-IM to perform all three functions concurrently with overlapping demands on the same signaling molecules. 5) In any kind of stretch in the gastric wall, the circular smooth muscle cells would stretch to the same degree as the ICC-IM. Several reports have demonstrated that the smooth muscle cells themselves express stretch-activated channels (87). It is not clear how the sensory signals from the ICC-IM would integrate with those from the circular smooth muscle cells. Is there a need for dual sensing of the stretch stimulus? Taken together, compared with the well-established sensory roles of the intrinsic neurons, extrinsic neurons, and smooth muscle cells, the feasibility of the ICC-IM in the gut to act as mechanoreceptors or the physiological significance of this sensory function appears to be low.

**ICC in Gastrointestinal Motility Disorders**

A reduction in the volume of the ICC or damage to their process has been reported in almost every clinical motility disorder that has been investigated, including Hirschsprung’s disease (89, 90, 173), chronic constipation (198), slow-transit constipation (7, 67, 189), megacolon (189), gastroparesis (199), diabetic mellitus (108), inflammatory bowel disease, animal models of inflammation (102, 122, 127), pyloric stenosis (92, 172, 195), achalasia (56, 61, 63), and chronic idiopathic intestinal obstruction (81, 156). However, there are little or no data to correlate the abnormalities of specific subtypes of ICC with specific features of motility dysfunction or clinical symptoms.

Note that almost all subtypes of ICC in one or more gut organs are absent, reduced in numbers, or have impaired processes in the W/W<sup>v</sup> mice. If the ICC regulated the generation and propagation of slow waves, mediated the excitatory and inhibitory neuronal input to the smooth muscle cells, and acted as mechanoreceptors, the motility function throughout
the gastrointestinal tract of these mice would be impaired, resulting in the symptoms of dysphagia, achalasia, altered gastric emptying, pyloric stenosis, altered small intestinal transit, constipation or diarrhea, and obstructed defecation. None of these clinical symptoms are noted in these mutants.

The animal models of some of the above motility disorders have provided further insights into the potential roles of the ICC in normal motility function as well as in motility dysfunction. The following sections discuss two of these experimental models.

Gastroparesis. Gastric emptying is slower, and slow-wave activity is impaired in patients with diabetic or idiopathic gastroparesis (27, 28, 85). The rate of gastric emptying is slower also in the NOD diabetic mice (118). The organ-bath studies show that the slow waves are present in some antral smooth muscle cells but not in others in the diabetic animals. The diabetic mice show reductions in the numbers of the ICC in discrete patches. The amplitudes of the EJPs and the IJPs are smaller in the NOD diabetic mice compared with those in the NOD non diabetic controls. However, other investigators have reported alterations in cell signaling, reductions of neurotransmitter release, and alterations of cellular excitability in diabetic animals, which are also likely to contribute to the observed alterations in slow-wave activity, junction potentials, and gastric emptying in diabetic models (163, 164, 191).

Partial luminal obstruction. The lethal spotted mouse (ls/ls) is an interesting model from the point of view of the ICC. A thorough study found that the distribution and the density of the different subtypes of ICC in ls/ls mice are not different from those in age-matched controls (185). However, the circular smooth muscle cells are electrically quiescent, and their postjunction responses are significantly reduced in the ganglionic segments of these mice. Interestingly also, the RMP in the aganglionic segments is not different from that in the normal segments, probably because both areas have intact ICC. The important message from this model is that the impairment of the slow-wave activity and reduction in postjunction responses can occur in smooth muscle cells by non-ICC related factors. These factors include a switch in the cell-signaling pathways or changes in the expression of cell-signaling proteins (146–148). The concurrent investigations of these alternate possibilities in patients and in animal models of disease are helpful in identifying correct etiologies of motility dysfunction.

The creation of experimental partial obstruction in the small intestine alters the structure and phenotype of the ICC oral to the site of obstruction so that they become more like myofibroblasts (26). This transformation occurs with decreasing intensity up to about 10 cm oral to the site of partial obstruction. The intensity of disruption of the ICC correlates with the decrease of the circular smooth muscle RMP and the slow-wave amplitude in the oral segment, which is consistent with the loss of RMP gradient.

However, there is a disconnect between the full recovery of the ICC, and the electrical characteristics of the smooth muscle cells and the generation of the postjunction potentials. The recovery of the slow-wave amplitude, the RMP, and the generation of postjunction potentials is only partial when the ICC have recovered fully. This suggests that the alterations in the microenvironment oral to the partial obstruction site that transform the ICC to fibroblasts have direct effects on the function of the smooth muscle cells and the enteric neurons.

The findings of the partial obstruction and small bowel resection are very revealing as to why the ICC are impaired in almost all diseases that cause motility dysfunctions. It seems that the plasticity of the ICC allows them to readily alter their phenotype to that of the myofibroblasts or the smooth muscle cells in response to changes in their microenvironment, such as that during hypoxia (30, 196) hypertrophy/hyperplasia, and an inflammatory response. This transformation of the ICC to fibroblasts or smooth muscle-like cells may occur as soon as within 5 h after intestinal resection (196). Reverse transformation of the ICC occurs as soon as the microenvironment returns to the normal state (26, 196).

Finally, the slow waves make important contributions to the generation and propagation of the RPCs (Table 1). However, three distinct types of contractions, the RPCs, the UPCs (GMCs and RGCs), and tone, together regulate the overall motility function (133, 140). The GMCs cause rapid mass movements, and they provide the rapid propulsive force to evacuate feces during defecation (133, 140). A decrease in the frequency of GMCs causes constipation (4–6, 9, 22, 154), whereas an increase in their frequency causes diarrhea (29, 31, 32). The RGCs are essential for rapid regurgitation from the proximal half of the small intestine to the stomach in preparation for vomiting (91). The generation of tone is essential for the function of the sphincters. The tone is increased also in the small intestine and the colon in the postprandial state. This increase in tone enhances the efficacy of RPCs by narrowing the lumen. The noteworthy thing is that the slow waves do not regulate the UPCs and the tone (Table 1). Consequently, the absence of the ICC would have little effect on the contributions of the GMCs, RGCs, and tone to the overall motility dysfunction. It is noteworthy also that the decrease in the amplitude and propagation of the slow waves would have the greatest effect on transit in the proximal small intestine and the stomach, where the slow waves are phase locked and propagate over longer distances. In the colon and the distal small intestine, the slow waves are largely phase unlocked and unstable in amplitude to begin with, and the alterations in their characteristics due to the damage to the ICC may be small (134–138, 140). These may be some of the reasons that the W/Wv mice with severe abnormalities of the ICC throughout the gastrointestinal tract do not exhibit major motility dysfunctions. It also emphasizes the need to investigate alterations in all three types of gut contractions in motility disorders to arrive at correct diagnosis and etiologies of motility disorders.

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GRANTS

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REFERENCES


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