The generation and propagation of gastric slow waves

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Summary

1. Mechanisms underlying the generation and propagation of gastrointestinal slow wave depolarizations have long been controversial. This review aims to collate present knowledge on this subject with specific reference to slow waves in gastric smooth muscle.

2. At present, there is strong agreement that interstitial cells of Cajal (ICCs) are the pacemaker cells that generate slow waves. What has been less clear is the relative role of primary types of ICCs including the network in the myenteric plexus (ICC-MY) and the intramuscular network (ICC-IM). It is concluded that both ICC-MY and ICC-IM are likely to serve a major role in slow wave generation and propagation.

3. There has been long-standing controversy as to how slow waves “propagate” circumferentially and down the gastrointestinal tract. Two mechanisms have been proposed, one being action potential-like (AP-like) conduction and the other phase wave-based “propagation” resulting from an interaction of coupled oscillators. Studies made on single bundle gastric strips indicate that both mechanisms apply with relative dominance depending on conditions; the phase wave mechanism dominant in circumstances of rhythmically generating slow waves and AP-like propagation dominant when the system is perturbed.

4. The phase wave mechanism (termed *Ca^{2+} phase wave*) utilises cyclical Ca^{2+} release as the oscillator with coupling between oscillators mediated by several factors including: i) store-induced depolarization; ii) resultant electrical current flow/depolarization through the pacemaker cell network and iii) depolarization-induced increase in excitability of downstream Ca^{2+} stores. An analogy is provided by pendulums in an array coupled together by a network of springs. These, when randomly activated, entrain to swing at the same frequency but with a relative delay along the row giving the impression of a propagating wave.

5. The AP-like mechanism (termed *voltage-accelerated Ca^{2+} wave*) propagates sequentially like a conducting action potential. However, it is different in that it depends on regenerative store Ca^{2+} release and resultant depolarization rather than regenerative activation of voltage-dependent channels in the cell membrane.

6. The applicability of these mechanisms to describing propagation in large intact gastrointestinal tissues, where voltage-dependent Ca^{2+} entry is also likely to be functional is discussed.

Gastric motility

Gastrointestinal motility is fundamentally dependent on rhythmic depolarizations termed slow waves (Figure 1). These rhythmically occurring depolarizations occur in gastric smooth muscle at low frequency (*e.g.* < 5/min), the depolarization activating voltage-dependent Ca^{2+} channels causing smooth muscle (SM) contraction. A key feature of slow waves is that they propagate relatively rapidly circumferentially (*e.g.* see Hirst, Garcia-Londono & Edwards, 2006[1]), squeezing the stomach and intestines, but spread more slowly in an oral-anal direction to appropriately direct gastric contents. Yet key aspects such as how slow waves propagate remain only partly understood. Such knowledge is fundamental to understanding and treating disabling motility disorders such as gastroparesis.

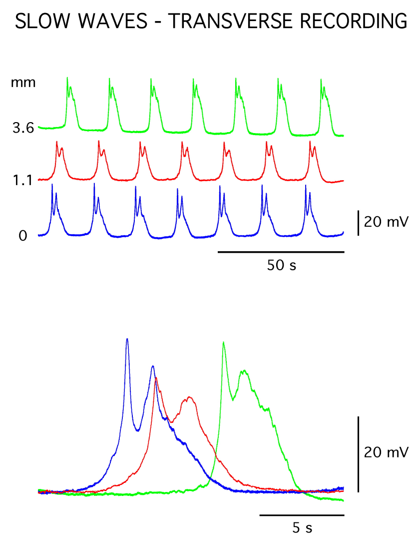

Figure 1. Slow waves recorded with intracellular micro-electrodes in a sheet of circular smooth muscle from the guinea pig distal gastric antrum. *The slow waves generated at a frequency of about 3/min and had initial and secondary depolarizing components. The slow waves exhibited propagation delays as demonstrated by comparison of simultaneous recordings made at three sites located transversely (*i.e. *oro-anally) across the circular muscle sheet (separations of 1.1 and 3.6 mm from the most orally located electrode). The average membrane potential of the tissue was −62 mV.*

The basis for slow wave generation has received

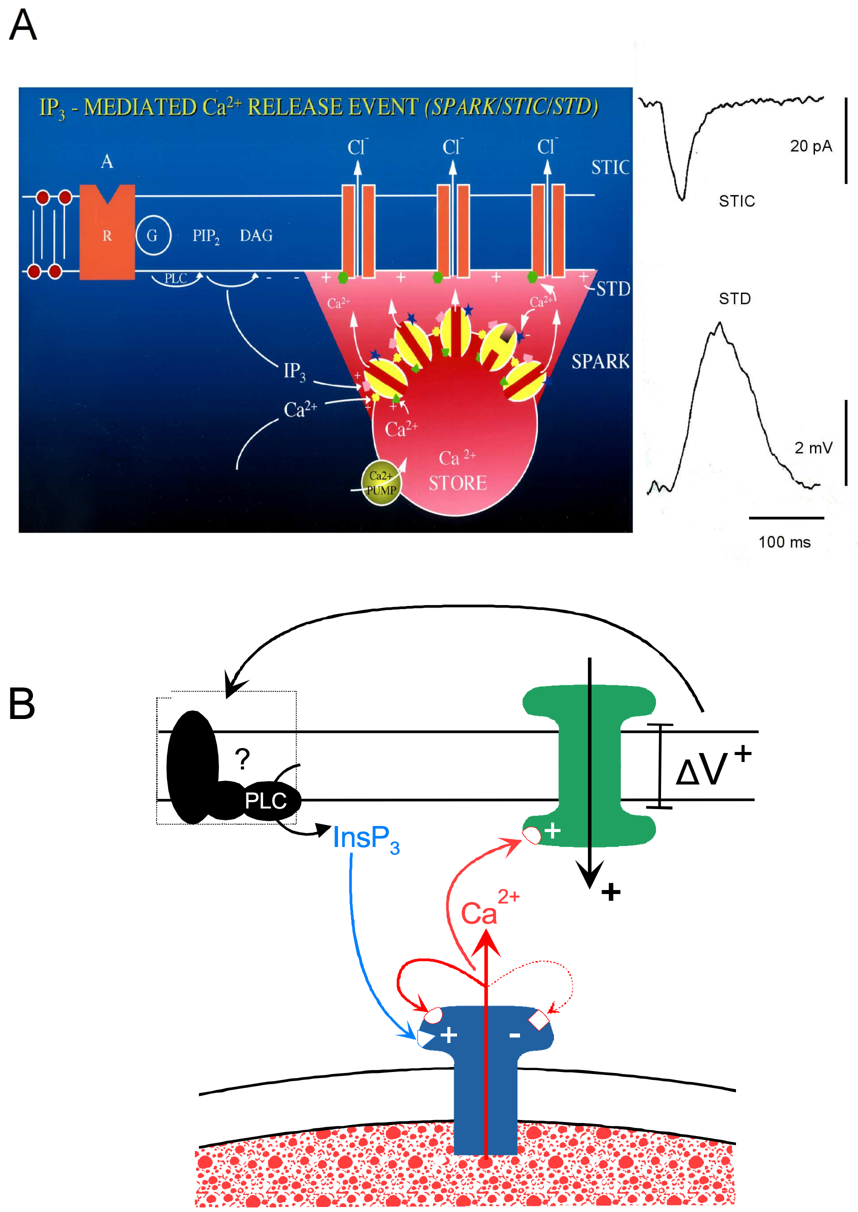

Figure 2. Model of Ca^{2+} store function in gastric ICCs. ***A:*** *Schematic depicting an intracellular SR/ER Ca^{2+} store located just below the cell membrane that cyclically releases Ca^{2+} into the cytosol through activation of IP_3Rs. While the resultant transient increase in $[Ca^{2+}]_c$ is shown to open Cl^- channels generating a spontaneous transient inward current (STIC) and resultant spontaneous transient depolarization (STD), it is to be noted that the role of Cl^- channels here remains controversial and while supported by some[39,40,43] has been proposed by others to be a non-selective cation channel.[41] Furthermore, while inward current generation has been associated with an increase in $[Ca^{2+}]_c$, there is also evidence for a second mechanism that reduction in $[Ca^{2+}]_c$ can open non-selective cation channels, as could occur during the store refill cycle and/or during Ca^{2+} uptake by mitochondria (see Takeda* et al.*, 2008[41]).* ***B:*** *Schematic depicting the proposed positive relationship between store Ca^{2+} release-induced membrane depolarization and resultant enhancement of Ca^{2+} release from IP_3R-operated Ca^{2+} stores. While, the presence of the voltage feedback mechanism is accepted, the mechanism by which membrane depolarization causes enhanced Ca^{2+} release remains controversial. The proposal shown here was based on studies on single bundle gastric strips where the feedback was shown to continue despite the presence of Co^{2+} or Cd^{2+} antagonists of voltage-dependent Ca^{2+} channels and could be blocked by NEM, which is known to prevent activation of specific G-proteins.[9,11,39] The voltage-dependent Ca^{2+} channel feedback proposal is based on findings in intact tissues such as the canine gastric antrum (see Hirst* et al.*, 2002[89]). Diagram A from van Helden & Imtiaz, 2003;[55] B from van Helden* et al.*, 2000.[11]*

longstanding interest, but a generally accepted mechanism for slow wave generation has only arisen over the last twenty years. This leap forward was based on two key findings. The first was the visionary suggestion[2] followed by experimental evidence[3-7] that pacemaking activity was generated in another gastrointestinal cell type termed Interstitial Cells of Cajal (ICCs), with the resultant electrical activity considered to passively transmit through gap junctions to the smooth muscle cells to open voltage-dependent Ca^{2+} channels and contraction. The second finding was that slow wave generation was mediated by store Ca^{2+} release and not by voltage-dependent channels.[8-12] This mechanism, as previously described from studies on lymphatic smooth muscle,[13] is generated by intracellular Ca^{2+} stores in their capacity to cyclically release and take up Ca^{2+}, the change in cytosolic Ca^{2+} concentration ($[Ca^{2+}]_c$) causing opening of ion channels in the cell membrane that carry inward current and depolarize the membrane (Figure 2).

ICCs as pacemaker cells

There are various types of ICCs that differ according to their location and/or morphological appearance.[14-18] Of these, the most dominant ICCs in the stomach occur in the myenteric plexus layer (ICC-MY) and intramuscularly within the muscle bundles (ICC-IM). ICC-MY (also referred to as ICC-AP[5,19]) and ICC-IM, while both detected by antibodies to the proto-oncogene Kit,[3,4] show very different morphologies, the former being stellate shaped and the latter spindle shaped.[17,20] Generally, ICC-MY are considered to act as pacemaker cells[3,4,6,7] with ICC-IM serving as a target for gastric innervation.[21,22] However, it seems possible that ICC-IM also have pacemaker capability. Evidence for this comes from several studies. Firstly, agonist stimulation causes a shift where ICC-IM rather than ICC-MY appear to become dominant in establishing pacemaking in the gastric antrum.[23] Secondly, isolated gastric antral/pyloric single bundle strips where there are only ICC-IM exhibit spontaneous slow wave-like activity.[9,24] Thirdly, gastric slow waves originate in the guinea pig gastric corpus, a region where there are ICC-IM but no ICC-MY cell networks.[25]

Slow waves often show two components, a "first" and "second" component[26] (Figure 1). A well-studied example of this is in the circular muscle layer of the gastric antrum. Here, evidence has been presented that the "first" or "initial" depolarizing component, termed "driving" or "pacemaker" potential, is generated in the network of ICC-MY[7] and the "second" component is generated by ICC-IM in the smooth muscle layer.[27] This latter proposal is supported by findings that gastric antral slow waves in W/W^V mutant mice, where ICC-IM are not present, do not exhibit this second component.[23] Furthermore, studies in control mice demonstrate that slow waves are composed of "initial" and "secondary" components nearer the greater curvature where there is a high density of ICC-MY but only a secondary component at the lesser curvature where ICC-MY are effectively absent.[23] These latter slow waves are similar in appearance to the single component slow waves of the gastric corpus where ICC-IM and not ICC-MY are present.[25] Slow wave-associated activity recorded from antral longitudinal muscle, termed "follower" potentials, appears to result through passive current flow from "driving" potentials generated in the ICC-MY network.[7] Importantly, antral ICC-MY, which lie between the two muscle layers, have been shown to be electrically coupled to both the circular and longitudinal muscle layers. Here coupling needs to be strong, allowing sufficient current flow from the ICC-MY network to generate follower potentials in the longitudinal smooth muscle and the initial component of slow waves in the circular smooth muscle.[28] The proposal that activity in antral longitudinal muscle is simply passive current spread from ICC-MY suggests that ICC-IM, while reportedly present at low density,[17] have no functional pacemaker role in the longitudinal muscle layer.

Slow waves may also vary in waveform because of activation of L-type Ca^{2+} channels, with some circular muscle slow waves or longitudinal muscle follower potentials exhibiting longer lasting depolarizations or superimposed L-type Ca^{2+} channel-mediated spike potentials.[26,29] It is not surprising that such activity should exist given that activation of L-type Ca^{2+} channels is an inherent property of slow waves without which slow wave-induced contractions do not occur. Indeed what is surprising is that some slow waves such as those of the guinea pig gastric antrum do not show spike potentials and show the same waveform even when L-type Ca^{2+} channels are blocked.[9,30] Presumably in the latter case the relative electrical contribution of the L-type Ca^{2+} channels is shunted by a much larger conductance of the channels that generate the slow wave potential. In this regard, there is long standing evidence that the second component of slow waves (*i.e.* that considered to be generated by ICC-IM) corresponds with an increase in membrane conductance (see Tomita, 1981[26]).

Cellular mechanisms underlying generation of slow waves

The first electrical recordings of slow waves led to these events being referred to as "action potentials of the stomach".[31] The mechanism was different to that of the nerve and skeletal muscle action potentials leading to a range of proposals involving ionic conductance mechanisms and/or ion transport mechanisms (see Tomita, 1981[26]). Slow waves were not traditional action potentials with activity resulting through regenerative activation of voltage-dependent ion channels. In contrast, slow waves exhibited both a regenerative component and a voltage-independent component most likely generated by intracellular mechanisms (see Tomita, 1981[26]).

The finding that intracellular inositol 1,4,5-trisphosphate receptor (IP_3) -operated Ca^{2+} stores provided a pacemaker mechanism provided resolution to these studies.[8-12] The mechanism operates as follows. Firstly, intracellular Ca^{2+} stores of the sarcoplasmic/endoplasmic reticulum (SR/ER) are oscillators cyclically releasing and re-uptaking Ca^{2+} with further modulation provided by mitochondria.[8-12] Stores and mitochondria that are both near the plasma membrane can substantially modulate local cytosolic $[Ca^{2+}]_c$ in the subplasmalemmal space[32] activating Ca^{2+} dependent channels in the cell membrane through either the rise or fall in $[Ca^{2+}]_c$ depending on the channel type. This mechanism provides the pacemaker "clock" cyclically activating slow waves according to the timing of the release-refill cycle of Ca^{2+} stores. Interestingly, the store pacemaker mechanism as studied in both freshly isolated tissues and cultured gastric cell networks relies primarily on IP_3R and not ryanodine receptor (RYR)-operated Ca^{2+} stores.[8-12]

In addition to providing the pacemaker mechanism, IP_3R-operated Ca^{2+} stores also underlie the regenerative component of slow waves. This finding has come from studies on single bundle strips of guinea pig antral smooth muscle.[9,11] The regenerative component corresponds to the second component of slow waves and is considered to be generated by ICC-IM.[27] It is comprised of summations of events termed "unitary potentials" or "spontaneous transient depolarizations".[11,33] Each event arises from localised release of Ca^{2+} from IP_3R-operated Ca^{2+} stores most likely within a single ICC-IM, as has been demonstrated for urethral ICCs.[34] The regenerative component of these single bundle strip "slow waves" or "slow potentials", as they are variously referred to,[11,35] represents near synchronous activation of many of these events. The process is regenerative because under sufficient levels of stimulation (*i.e.* when the $[Ca^{2+}]_c$ or $[IP_3]$ is sufficiently high) the release of Ca^{2+} of one or a few of these events activates further Ca^{2+} release events in a regenerative manner. This process was originally described for RyRs, a process referred to as Ca^{2+}-induced Ca^{2+} release (CICR)[36] but equally applies to IP_3Rs.[37] Thus in overview, slow waves in single bundle gastric strips are each composed of a pacemaker component caused by the Ca^{2+} release/refill cycle of active IP_3R-operated stores and a resultant triggered regenerative activation of previously quiescent IP_3R-operated stores. It has yet to be determined whether or not these are two distinct populations of Ca^{2+} stores.

Generation of slow waves in the gastric corpus, where there are only ICC-IM, is likely to result through this same mechanism.[25,38] However, the mechanisms generating slow waves recorded in the gastric antrum nearer the greater curvature are more complicated as they also involve ICC-MY. Here, the second component of slow waves, which is likely to be driven by ICC-IM involves the same mechanism (*i.e.* regenerative IP_3R-mediated Ca^{2+} release).[27]

Detailed studies on the first component have been made by direct recording of "driving" potentials, otherwise referred to as "pacemaker" potentials, from ICC-MY. These potentials are themselves composed of two components with the primary component likely to be generated by voltage-dependent Ca^{2+} channels and the second component generated by release or uptake of Ca^{2+} from IP_3R-operated Ca^{2+} stores,[39-41] which activates inward current the ionic basis of which remains controversial (see below). Interestingly, both components are able to operate when the other is inhibited indicating they are not co-dependent.[39,40] Studies on cultured murine intestinal ICCs also report a voltage-dependent component, which pharmacological studies indicated were carried by voltage-dependent T-type Ca^{2+} channels.[42] The second component of the pacemaker potential and the antral circular muscle slow wave (*i.e.* presumably that generated by ICC-IM) have been proposed to be generated by activation of Cl^- channels as both showed a sensitivity to Cl^- channel blockers[39,40] (see also Huizinga *et al.*, 2002[43]). There is also histochemical evidence that Ca^{2+} activated Cl^- channels are present in ICCs.[44] In contrast, studies on cultured[45] and freshly isolated[46] ICCs from the murine small intestine indicate that this current is carried by a cationic current that opens with depletion of cytosolic Ca^{2+} concentration ($[Ca^{2+}]_c$). The characteristic stellate shape of ICC-MY as opposed to the spindle shape of ICC-IM[17,20] of the freshly isolated ICCs and the location near the nerve plexus indicates these were ICC-MY.[46] A recent study on freshly dispersed ICC from the murine gastric antrum that contained both ICC-MY and ICC-IM found that ICCs, identified by PCR as being positive to Kit, exhibited either a basal non-selective cation conductance (NSCC) that was inhibited by increase in $[Ca^{2+}]_c$ or a Ca^{2+}-activated NSCC.[41] Comparison of cell morphology suggested that the basally active NSCC was present in ICC-MY and the Ca^{2+}-activated NSCC was present in ICC-IM as the corresponding cell morphologies were stellate and spindle shaped respectively. Significantly, Cl^- channel blockers inhibited the Ca^{2+}-activated NSCC current.[41] However, this said, it is now known that ICCs express the Ca^{2+}-activated Cl^- channel, ANO1 and it has recently been shown that this current is linked to slow wave currents and pacemaker activity.[47]

In overview, the field seems to be in general agreement as to the key role of ICCs and ER/SR intracellular IP_3R-operated Ca^{2+} stores in pacemaking and generation of the dominant components of slow waves in gastric circular muscle.[8-12,48] In tissues such as the guinea pig gastric corpus and antrum near the lesser curvature slow waves are monophasic and initiated by ICC-IM.[23,25] In the gastric antrum nearer the greater curvature slow waves are biphasic, the first component reflecting passive current flow from "driver" potential activity in ICC-MY and the second component paralleling that of the gastric corpus (*i.e.* dependent on ICC-IM).[7,23,26,27] Direct recording from ICC-MY *in situ* demonstrates that the "driver" potentials in these cells are themselves biphasic, demonstrating a brief initial transient most likely carried by nifedipine-resistant voltage-dependent Ca^{2+} channels and a longer sustained component.[7,23,40] While there is agreement that this second "driver" potential component is generated by interplay between IP_3R-operated stores and mitochondria, the conductance mechanism is controversial. Studies on cultured and freshly isolated ICCs suggest that the "driver" potential conductance is a NSCC activated by decrease in $[Ca^{2+}]_c$[41,45,46] rather than a Ca^{2+}-activated inward current.[40] However, the finding that the rapid Ca^{2+} chelator BAPTA/AM or Ca^{2+} free solution inhibits the "driver" potentials[40] suggests that the channels are Ca^{2+} activated rather than Ca^{2+} inhibited. Therefore, while Ca^{2+}-inhibited channel activation mechanism remains a highly interesting proposal, one for which there are clearly candidate channels (*e.g.* TRP channels; see Takeda *et al.*, 2008[41]), the role of this mechanism in generation of pacemaker potentials in guinea pig ICC-MY remains controversial. In contrast, there is a compendium of evidence that Ca^{2+}-activated Cl^- channels have a significant role in generation of slow waves.[39,43,47]

Coupled oscillator-based entrainment of Ca^{2+} stores – a requisite component of slow wave generation

Ca^{2+} store-mediated pacemaking can only be functional if there is coordination of the cycling of the Ca^{2+} stores within and/or between coupled cells, as Ca^{2+} stores generate far too little current to individually pace the tissue.[13,49,50] Stores achieve this by interacting as coupled oscillators, which entrain when there is sufficient coupling force between the oscillators.[24,51-57] The mechanism can be readily illustrated using the analogy of an array of pendulums coupled together by interconnecting springs (Figure 3A,B). These, when randomly activated, push or pull their neighbours *via* the spring connections causing the pendulums to entrain. Such entrainment can be near synchronous between local groups of adjacent pendulums but this will depend on the relative strength of the coupling (*i.e.* the springs).

Ca^{2+} stores and pendulums differ in that, while swinging pendulums exhibit the same period forward and back, Ca^{2+} stores show a release period that can be very different to the refill period. Such oscillators are termed "relaxation" oscillators. A simple analogy is the tipping urn common to Japanese gardens that, upon filling, tips and spills its contents, then uprighting and refilling. Here the event interval is dependent on the size of the urn and the rate of refill. The cycling rate of Ca^{2+} stores can vary enormously and is dependent on factors including the size of each store, the rate of refill of the Ca^{2+} store, the threshold for triggering release and the rate of Ca^{2+} release. Stores refill through the sarco/endoplasmic reticulum calcium ATPase (SERCA). They divest their contents through the transient opening of either IP_3Rs and/or RyRs, Ca^{2+} release channels that span the membrane of the SR/ER Ca^{2+} store (see Takeda *et al.*, 2008[58]). The threshold for such opening is set by both the SR/ER luminal Ca^{2+} concentration, cytoplasmic Ca^{2+} concentration and the level of cytosolic activators, the latter including $[Ca^{2+}]_c$ for both IP_3Rs and RyRs and IP_3 for IP_3Rs. Higher cytosolic levels of these activators make the stores more excitable, functionally reducing the luminal Ca^{2+} concentration ($[Ca^{2+}]_L$) at which the Ca^{2+} release channels open. Therefore, Ca^{2+} store cycling and hence pacemaker frequency is readily altered simply by changing levels of cytosolic activators.

Stores can only subserve a pacemaker mechanism if there is sufficient entrainment to drive the syncytium of pacemaker cells. To achieve this, stores must be strongly coupled both within and between cells. A primary coupling mechanism between stores is the diffusion of Ca^{2+} and resultant CICR. However, this coupling mechanism and variants thereof, which include diffusion of other store Ca^{2+} release activators (*e.g.* IP_3) are not strong and, while being able to induce weak within-cell entrainment,[51] cannot provide coupling sufficient for global within- and across-cell entrainment. In contrast, voltage coupling, whereby depolarization causes enhanced store activity by increasing Ca^{2+} entry or enhancing production of store activators such as IP_3, represents a much stronger coupling mechanism.[24,54] This is because voltage coupling has some 500 fold greater spatial intercellular influence than do diffusing ions. Thus, while Ca^{2+} release from any one region can only activate adjacent regions due to the effective diffusion distance of Ca^{2+} being only about 5 μm,[59] the depolarization caused by local Ca^{2+} release can readily transmit by current flow across the syncytium of coupled cells. Such coupling will therefore have a much more global influence on stores, which become activated by depolarization-induced Ca^{2+} entry and resultant CICR-induced activation of IP_3Rs, as occurs in lymphatic and blood vessels[52,54,56,57] or by depolarization-induced enhancement of IP_3R, as has been proposed to occur in gastric tissues.[24,56,57,60]

Depolarization-induced Ca^{2+} release

As outlined above, a positive link between membrane depolarization and store Ca^{2+} release is a fundamental prerequisite for entrainment of Ca^{2+} stores and resultant pacemaking. However, while depolarization-induced Ca^{2+} entry is an accepted link mechanism, the basis for depolarization-induced enhancement of IP_3R-mediated Ca^{2+} release as reported in gastric tissues remains in contention. Studies on this subject have primarily been made using single bundle gastric strips, tissues in which there are ICC-IM but not ICC-MY. Depolarization of these tissues triggers regenerative depolarizations that commence with a delay of at least 1 s and that are caused by IP_3R-mediated Ca^{2+} release.[9,11,12,39] They are unlikely to be caused by voltage-dependent Ca^{2+} entry as they persist in the presence of Co^{2+} or Cd^{2+} -containing physiological saline solutions.[9,11] Furthermore, while, L-type Ca^{2+} channels permit additional Ca^{2+} entry during this regenerative response, the amplitude of the regenerative depolarization occurs independent of these channels.[39] The response is also absent when IP_3Rs are blocked by heparin,[11] in antral muscle from a mouse mutant where there are no Type 1 IP_3Rs and in the presence of 2-APB an agent known to both block IP_3Rs and inhibit store refill.[12,61] Recordings made when IP_3Rs are absent or blocked demonstrate little evidence of spontaneous Ca^{2+} release indicating that even under resting conditions the mechanisms initiating spontaneous Ca^{2+} release are active. Such activity is likely to be generated in ICC-IM, as spontaneous activity is not present in W/W^V mutant mice where ICC-IM are absent.[27]

Taken together, the observation that depolarization enhances IP_3R-mediated Ca^{2+} release in gastric tissue strips cannot be explained in terms of conventional voltage-dependent Ca^{2+} entry and resultant CICR, though Ca^{2+} dependence is not excluded. Experimental findings investigating this feedback mechanism provide support for the hypothesis that depolarization enhances production of

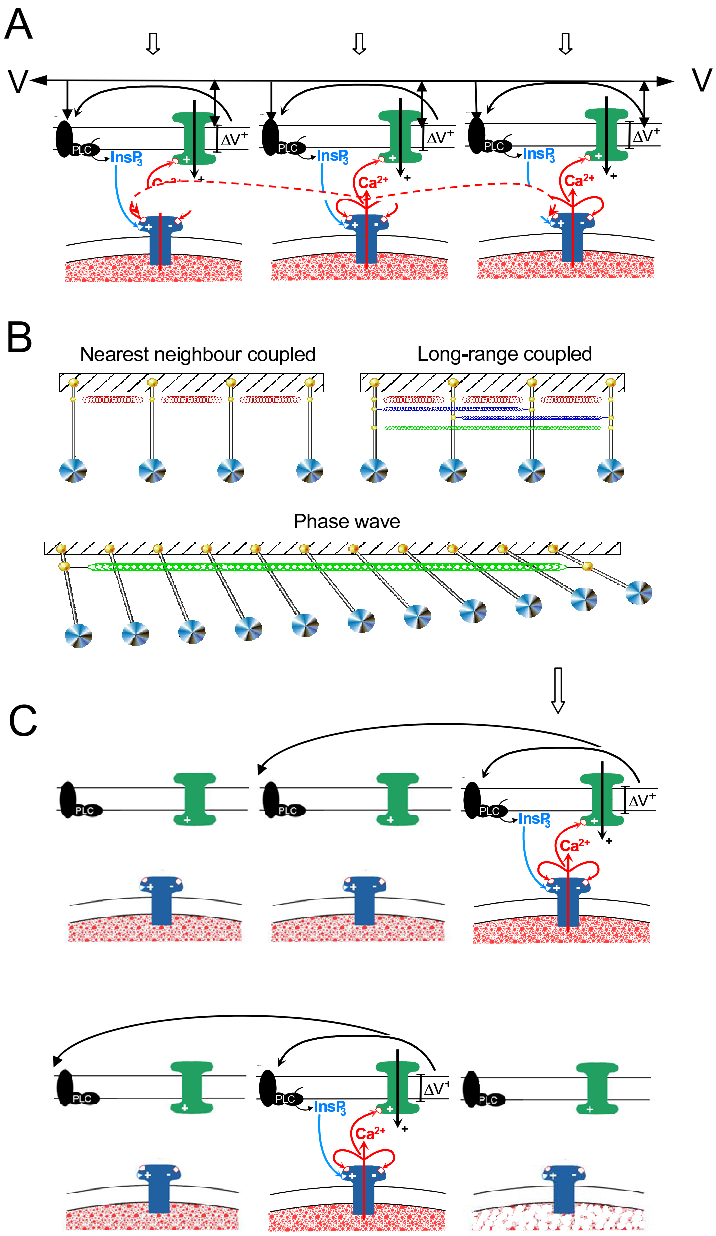

Figure 3. The coupled oscillator Ca^{2+} slow wave model incorporating voltage feedback occurs through generation of IP_3 or Ca^{2+} entry and resultant CICR and membrane depolarization. A: *Illustration showing that a low level of global stimulation (*e.g. *by an IP_3R agonist; block arrows) leads to oscillatory Ca^{2+} release (N.B. this can also occur through endogenous activation of stores as it does in slow-wave exhibiting gastrointestinal tissues). The oscillations from each Ca^{2+} store are coupled by either diffusion of Ca^{2+} (short-range diffusional coupling) or by spreading of the surface membrane potential (long-range voltage coupling).* ***B:*** *Ca^{2+} oscillators epicted here as pendulums connected by short-range nearest neighbour coupled springs (*i.e. *diffusional coupling by Ca^{2+} and/or IP_3) and long-range coupled springs (*i.e. *voltage coupling by intercellular spread of membrane current). Short-range coupling is too weak to be effective whereas long-range coupling has the strength to entrain large arrays of oscillators. However, while these oscillate at the same frequency there are small delays between the oscillators. This builds up along the array giving the impression of a propagating wave but this is, in fact, a phase wave generated by oscillators, as depicted by the array of pendulums (diagram from van Helden & Imtiaz, 2003[55]).* ***C:*** *Shows how the same coupling processes can lead to a* voltage-accelerated Ca^{2+} wave *in response to a strong localised stimulus (*e.g. *local membrane depolarization). The propagation of this Ca^{2+} wave relies on sequential depolarization of the surface membrane voltage, which then initiates the regenerative Ca^{2+} store- mediated-feedback loop. This wave cannot be activated in tissue regions where stores are spent and hence can only propagate when stores are excitable and does not back-propagate (C, lower record).*

IP_3. How this occurs is unknown but there are precedents for this hypothesis. For example, direct measurement of $[IP_3]$ in vascular smooth muscle before and during hyperpolarization indicate that $[IP_3]$ is decreased with hyperpolarization.[62] Other findings include: depolarization-induced enhancement of inositol phosphate levels in jejunal longitudinal smooth muscle[63] and depolarization-induced enhancement of IP_3R-mediated Ca^{2+} release in skinned skeletal muscle cells,[64] coronary myocytes[65] and megakaryocytes.[66] Another experiment addressing the issue of voltage-dependent production of IP_3 in gastric antral strips found that the voltage-dependent response was abolished by N-ethylmaleimide (NEM). This was a specific action as it did not block spontaneous depolarizations, but blocked the membrane depolarization-induced recruitment of these events, summations of which underlie the regenerative response.[39] One of the known actions of NEM is to prevent activation of specific G-proteins.[67,68] There is also evidence that membrane proteins such as phosphoinositide phosphatases, members of the protein tyrosine phosphatase superfamily, are coupled to an intrinsic voltage sensor.[69-71]

Importantly, studies on single bundle strips from murine and guinea pig gastric fundus demonstrate a complete absence of the depolarization-triggered regenerative response. This is not due to an absence of either ICC-IM or underlying spontaneous activity as the tissue is replete with ICC-IM[17,18] and demonstrates apparently normal spontaneous depolarizations, an activity that is not present in fundal strips from W/W^V mice where there are no ICC-IM.[72] Thus a key element is likely to be missing from ICC-IM in the gastric fundus, one that provides the link between membrane depolarization and store Ca^{2+} release. The absence of this link means that voltage coupling will be absent in the fundus with the consequence that stores will not synchronize and so theoretically cannot entrain their release-refill cycling to serve as a pacemaker mechanism.[24,55,56,73] This provides a simple explanation into the fact that the gastric fundus, or at least parts thereof,[74] do not exhibit slow waves and associated rhythmical contractions.

Slow wave propagation

Coupled oscillator versus core conductor generic models for slow wave conduction

Gastric slow waves and associated contractions are generally considered to first generate in the corpus of the stomach spreading in an oral-anal direction through the antrum to the pylorus.[25,75-78] In contrast, a recent study made in the canine stomach reported this site as the upper part of the fundus.[74] Studies on isolated strips of gastrointestinal muscle demonstrate that slow waves exhibit an oral-anal frequency gradient with the frequency decreasing in the oral-anal direction.[78,79] For example, strips of circular smooth muscle from the guinea pig gastric corpus exhibit slow waves in the frequency range of 4-6/min compared to a range of 3-5/min in the gastric antrum.[25,35] These and other observations led to adoption of a proposal originally made for the heart[80] that propagation of gastrointestinal electrical activity occurred through a coupled oscillator-based mechanism.[81-83] Such propagation has the appearance of a sequentially conducting wave (*e.g.* a conducting action potential) but in reality is a *phase wave* established by oscillators cycling at the same frequency but with a progressive phase delay along the array, as is exemplified for the array of pendulums in figure 3B. An alternative to the coupled oscillator model was the proposal that gastrointestinal electrical activity propagated as an action potential in a core conductor model.[84,85] This was an important challenge, as it highlighted several key issues that had not been addressed in the coupled oscillator model including: 1) that there was no definitive mechanism that could serve as the oscillator and; 2) there was no account of the core conductor properties of the smooth muscle/pacemaker cell syncytium.

Ca^{2+} phase waves and voltage-accelerated Ca^{2+} waves – Are these the mechanisms by which slow waves propagate?

The finding that Ca^{2+} stores provide the pacemaker mechanism for generation of slow waves and that this involves voltage feedback presented a means to progress the phase wave model. This was because: 1) Ca^{2+} stores are relaxation oscillators that can entrain by interacting as coupled oscillators; and 2) Ca^{2+} stores can only globally entrain to act as a pacemaker mechanism if they are strongly coupled, a mechanism requiring intercellular current flow.[24] Thus the two key "missing links" were fulfilled in that there was now a physical oscillator and a requirement for electrical conduction.

The existence of discrete networks of pacemaker cells (*i.e.* ICCs) that drive the smooth muscle adds further complexity to the system but does not necessarily change the model for slow wave propagation. It is likely that Ca^{2+} stores in pacemaker cells are dominant.[24] However, whether the resultant current flow passively drives the smooth muscle and/or also recruits Ca^{2+} stores in the smooth muscle needs further investigation. There is also controversy as to how intercellular current flow is mediated within and between the networks of pacemaker and muscle cells. This is because, intercellular current flow, while traditionally considered to be mediated by gap junctions, may also conduct through mechanisms such as field and/or metabolic coupling[19] due to adjacent cells often exhibiting membrane regions in close apposition but without gap junctions (*e.g.* see Garfield, 1985[86]). Indeed a recent study on retinal neuroepithelial cells provides evidence that intercellular current flow can occur through capacitative coupling currents generated by high-frequency fluctuations in the Ca^{2+} store potential.[87]

Coupled oscillators will entrain providing the coupling is sufficiently strong. However, even voltage coupling which is some 500 times more effective than diffusion (see above) is still limiting and, while allowing the Ca^{2+} stores to entrain near synchronously in local regions, marked phase delays between the oscillators can develop over larger distances as the coupling is not infinitely strong (*e.g.* distances > the tissue length constant which is 2 – 3 mm for single bundle antral/pyloric strips[24,1]). This, as has been discussed, gives the appearance of a propagating wave but is in fact a phase wave, the result of Ca^{2+} stores undergoing oscillatory Ca^{2+} release-refill at the same frequency but exhibiting a progressive phase delay along the array of stores (Figure 3 A,B).

The key question is whether there is experimental proof that Ca^{2+} stores serve this mechanism. The answer is yes but with qualification. The definite 'yes' component of this answer is based on studies on single bundle strips from the pyloric/distal antral region of the guinea pig stomach.[24] These strips exhibit propagating slow wave activity. The first experimental proof came from examining the effects of interrupting gap junction connectivity centrally along the strip. This was achieved by applying a narrow stream (~0.5 mm) of physiological saline solution (PSS) containing an agent, known to block gap junctions (*i.e.* 18–βGlycerrhetinic acid), centrally across the tissue strip. This decoupled the slow waves on the two sides of the tissue, both sides continuing to exhibit slow waves but at different frequencies and hence with no phase correspondence. This confirmed that the slow waves were interacting as coupled oscillators. A follow up experiment aimed to determine if Ca^{2+} stores were the mechanism for this coupling and if the coupling involved voltage coupling, as compared to coupling by diffusion (*e.g.* of Ca^{2+} or IP_3). This experiment used the same approach as the gap junction blocking experiment, but now using substances known to inhibit Ca^{2+} store function (*i.e.* caffeine; 2-APB). Decoupling was obtained with both these inhibitors supporting the postulate that Ca^{2+} stores were the underlying oscillator. Importantly, unlike application of the gap junction blocker (*i.e.* loss of connectivity) the Ca^{2+} store inhibitors required a much wider stream (width > 5 mm). As diffusion of Ca^{2+} or IP_3 has a very low effective coupling distance (*i.e.* <0.02 mm[59]), diffusion could not be the coupling mechanism. Rather, the large coupling distance is consistent with stores being coupled by voltage coupling, as the electrical length constant of these isolated strips was about 3 mm. Taken together these finding support the hypothesis that slow wave propagation in these strips occurs through phase delays established by long-range coupled oscillator-based interactions of Ca^{2+} stores. This means of propagation has been termed *Ca^{2+} phase waves*, as first it is a phase wave and second, as for sequentially conducting Ca^{2+} waves,[88] it has as its basis store Ca^{2+} release.[24,55]

Importantly, the mechanism whereby voltage-feedback causes IP_3R-mediated store Ca^{2+} release allows slow waves to also propagate sequentially through events we term *voltage-accelerated Ca^{2+} waves*.[24,55] These are the same as conventional Ca^{2+} waves but differ in that now the spread is not limited by the diffusion of Ca^{2+} in relation to conduction by CICR, but is mediated by current spread that, as considered above, has a spatial influence some 500 times greater than for the diffusion of Ca^{2+} (Figure 3C). Therefore, slow wave propagation in these single bundle strips is likely to occur by Ca^{2+} phase waves and/or voltage-accelerated Ca^{2+} waves. It is yet to be determined how the two mechanisms interact but it might be expected that Ca^{2+} phase waves dominate in a highly rhythmic system whereas voltage-accelerated Ca^{2+} waves will be increasingly important as store coupling becomes weaker and will become the dominant mechanism when slow waves are artificially stimulated out of synchrony (*e.g.* by locally-applied electrical field stimulation). However, it is to be noted that such artificial stimulation can only occur when the Ca^{2+} store population involved in normal pacemaking is not in a refractory state (*i.e.* where the Ca^{2+} stores have recently released their contents and without further store refill are unable to undergo further release), as is the case immediately after a slow wave. We predict that voltage-accelerated Ca^{2+} waves can propagate at rates as high as that established by Ca^{2+} phase waves providing they are evoked near to the peak of Ca^{2+} store excitability (*i.e.* at a frequency near to that of the spontaneously occurring slow waves) but this rate diminishes as the frequency of stimulation is increased (Imtiaz & van Helden, unpublished). In this regard, it is to be noted that the conduction velocity of stimulated slow waves is decreased with higher frequency stimulation.[89] The fact that these two mechanisms co-exist provides explanation into how both the generic coupled oscillator model and core conductor models could both have arisen and have generated such interesting debate (see Daniel *et al.*, 1994;[78] Publicover & Sanders, 1989;[84] Publicover, 1985[85]).

The Ca^{2+} phase and voltage-accelerated Ca^{2+} wave models for slow wave conduction considered so far have arisen from studies on single bundle gastric strips. The next step will be to see if these can be generalised to intact tissues assessing the relative roles of voltage-dependent channels, mitochondria and the role of different classes of ICCs. While a general consensus view on all these issues has still to be formed, some recent studies provide helpful insights. One group of studies[89-91] examined slow wave characteristics and propagation in strips of circular muscle from the canine gastric antrum using baths divided into two or three chambers. Experiments were made on spontaneous and triggered slow waves, the latter produced by electrical field stimulation (EFS) applied at a frequency slightly higher than the natural slow wave frequency. It was shown that neither TTX nor atropine had any effect on slow wave conduction velocity ("CV") (see also Nakayama *et al.*, 2006[30]). Temperature (T) had a large effect with the "CV" exhibiting a high Q_{10} of ~6 with T to < 24 ºC blocking propagation. Pharmacological investigations using a range of agents (*e.g.* the IP_3R and store-operated Ca^{2+} entry blocker 2-APB; the mitochondrial blockers Antimycin A, CCCP or FCCP; T-type Ca^{2+} channel antagonists Ni^{2+} and mibefradil) proportionally reduced the slow wave upstroke rate and "CV", whereas the L-type Ca^{2+} channel antagonist nifedipine or nicardipine had no effect. These data are consistent with the proposal that intracellular Ca^{2+} stores and mitochondria are integral to slow wave propagation. The data also suggest a role for T-type voltage-dependent Ca^{2+} channels with the authors suggesting that this mechanism, in its capacity to allow Ca^{2+} entry and resultant

CICR-based activation of IP_3Rs, provides the voltage-dependent feedback necessary for slow wave generation and propagation.[91] Thus this model does not rule out the Ca^{2+} phase wave/voltage-accelerated wave models made on single bundle strips from the guinea pig distal antrum,[24] but differs in the proposed voltage-dependent means by which stores are coupled. Indeed, it may be that in intact tissues from large animals such as the canine gastric antrum that coupling needs to be enhanced by voltage-dependent Ca^{2+} entry for slow waves to effectively propagate (*i.e.* stronger springs between oscillators; see Figure 3).

An interesting additional finding was that disabling of Ca^{2+} stores by the SERCA inhibitor CPA (50 μM) blocked the slow wave second component but did not block propagation of the first transient component.[89] This suggests that when stores are directly disabled by blocking the SERCA in circular muscle of the canine gastric antrum, action potential-like conduction can now occur through voltage-dependent channels. In comparison, slow wave propagation is abolished when intracellular Ca^{2+} homeostasis is interfered with by disrupting mitochondria and hence presumably IP_3R-operated stores or when the IP_3R inhibitor/store refill blocker 2-APB is used.[90] These differences need to be resolved. However, the relative role of voltage-dependent channels may be dependent on ICC-MY, as these channels are not obviously functional in tissues where there are ICC-IM but no ICC-MY (*e.g.* isolated single bundle gastric strips from the guinea pig gastric antrum; gastric pylorus or gastric corpus.[9,11,25,38] Indeed, the relative role of ICC-MY and ICC-IM in slow wave propagation remains controversial. For example, even in regions where ICC-MY are present (*e.g.* greater curvature of antrum) slow waves propagate at the same rate circumferentially along the circular SM when ICC-MY are removed by fine dissection.[1] This rate is 4-5 times faster than in networks of ICC-MY isolated from the circular SM but still connected to the longitudinal SM.[1,92] As a consequence, the much slower oro-anal conduction was considered to be carried by ICC-MY but the role of ICC-IM in transverse conduction was not tested.[1] The fact that slow waves in the guinea pig corpus, where there are ICC-IM but no ICC-MY, conduct both circumferentially and oro-anally[25] indicates that ICC-IM can also have a role in oro-anal conduction.

Conclusions

This review has considered current models for slow wave generation and propagation in the stomach. There is strong agreement that ICCs are fundamental to slow wave generation. However, what has been more controversial is the relative role of primary types of ICCs namely, ICC-MY and ICC-IM. It is concluded that ICC-IM are likely to serve a major role in slow wave generation and propagation, a role that has previously been underestimated. The controversy of how slow waves propagate has been reviewed. Evidence provided from studies on single bundle strips from the distal gastric antrum support the long held coupled oscillator model, the underlying oscillator being cyclical Ca^{2+} release from intracellular Ca^{2+} stores with coupling primarily mediated by membrane voltage. However, this model is probably simplistic for large intact gastrointestinal tissues, as while the model considered cellular coupling and resultant core conductor properties, the cells of the tissue did not obviously demonstrate voltage-dependent Ca^{2+} entry and hence this was not modelled.[24] In contrast, large intact gastric tissues containing both ICC-MY and ICC-IM demonstrate voltage-dependent Ca^{2+} entry, and models of slow wave propagation should account for this. The simplest view of this is that it would strengthen the coupling between stores making the Ca^{2+} store coupled oscillator mechanism even more effective. The alternative, is that the voltage-dependent channels take over, slow waves now conducting as action potentials, though the evidence for this so far only seems to apply for a special circumstance where store function is inhibited. Finally, to make matters even more intriguing, mechanisms underlying the Ca^{2+} store coupled oscillator model can also generate sequentially conducting waves. These share parallels to the conducting action potential model except that the propagating mechanism is the depolarization caused by the regenerative Ca^{2+} release that underlies the slow wave. This mechanism could explain propagation for electrically induced slow waves, events that are readily triggered just before the onset of spontaneously occurring slow waves.

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