A Model of Slow Wave Propagation and Entrainment Along the Stomach

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Abstract—Interstitial cells of Cajal (ICC) isolated from different regions of the stomach generate spontaneous electrical slow wave activity at different frequencies, with cells from the proximal stomach pacing faster than their distal counterparts. However, in vivo there exists a uniform pacing frequency; slow waves propagate aborally from the proximal stomach and subsequently entrain distal tissues. Significant resting membrane potential (RMP) gradients also exist within the stomach whereby membrane polarization generally increases from the fundus to the antrum. Both of these factors play a major role in the macroscopic electrical behavior of the stomach and as such, any tissue or organ level model of gastric electrophysiology should ensure that these phenomena are properly described. This study details a dual-cable model of gastric electrical activity that incorporates biophysically detailed single-cell models of the two predominant cell types, the ICC and smooth muscle cells. Mechanisms for the entrainment of the intrinsic pacing frequency gradient and for the establishment of the RMP gradient are presented. The resulting construct is able to reproduce experimentally recorded slow wave activity and provides a platform on which our understanding of gastric electrical activity can advance.

Keywords—Smooth muscle cell, Interstitial cell of Cajal, Electrophysiology, Resting membrane potential, Carbon monoxide.

ABBREVIATIONS

RMP Resting membrane potential
ICC Interstitial cells of Cajal
SMC Smooth muscle cell

INTRODUCTION

In recent years a number of simulation studies describing gastrointestinal (GI) tissue and organ electrophysiology have started to appear in the literature.1,2 These have generally adopted a bidomain approach, or its reduced monodomain form, as has been widely used in the cardiac field. However, the cell models that have been incorporated into these tissue descriptions have been phenomenological in nature and do not attempt to describe the underlying biophysics.1 Previously our group has developed biophysically based cellular electrical descriptions of the two key cell types involved in the generation and propagation of slow waves in the GI tract, the interstitial cells of Cajal (ICC) and smooth muscle cells (SMC).6,7 It is, however, non-trivial to combine these descriptions into a multi-cellular representation of tissue level slow wave activity. The motivation for this study is twofold; first to describe how to entrain an ICC to a voltage signal such as a slow wave, and second to introduce a method by which a resting membrane potential (RMP) gradient can be included in a tissue level simulation. The feasibility of these two methods is demonstrated through their incorporation into a dual-cable tissue level description of the gastric musculature.

In the intact stomach the ICC combine to develop a wave of electrical activity that propagates down the stomach, coordinating the mechanical contractions that generate motility. However, muscle strips isolated from the stomach generate slow waves at different regions of the stomach.
frequencies with the intrinsic pacing frequency being higher in the corpus than in the antrum. When the stomach is considered as a whole, the higher frequency corporal slow waves are able to entrain the lower frequency antrum giving rise to a dominant frequency throughout the stomach. At present, the underlying cause of this chronotropicity has yet to be experimentally determined and well-developed theories are lacking. The frequency at which an ICC initiates slow waves does, however, appear to be dependent on the rate at which intracellular Ca\(^{2+}\) is cycled among the endoplasmic reticulum, where it is released via IP3 receptor dependent channels, the pacemaker subspace, and the mitochondria where the uniporter sequesters the Ca\(^{2+}\) before it returns to the endoplasmic reticulum via the mitochondrial Na\(^+\)/Ca\(^{2+}\) exchanger and the SERCA pumps on the ER (depicted by the solid grey arrows in Fig. 1). To alter the intrinsic pacing frequency it would therefore appear that the rate of intracellular Ca\(^{2+}\) cycling needs to be altered.

Two main types of ICC have been identified in the stomach. The ICC-MY constitute a dense cellular network in the myenteric plexus, whereas intramuscular ICC (ICC-IM) are interspersed between SMC, mainly in the circular layer, in all regions of the stomach. Previously, the ICC-MY were widely believed to be responsible for the regular electrical slow wave activity in the stomach; however, now it appears that ICC-IM may also play a role. A diversity of slow wave activity in different regions of the stomach has been recorded experimentally by a number of investigators. The stomach fundus is more depolarized than any other region of the stomach and, despite displaying a dense population of ICC (mainly ICC-IM), is electrically silent in some species but is capable of generating slow waves in others. Higher amplitude slow waves appear in the mid-corpus region of the stomach near the greater curvature and propagate aborally toward the pylorus, which constitutes an electrical barrier to slow wave propagation. If one is interested in far-field simulations to produce either electrogastrograms or magnetogastrograms, then this heterogeneity should be included as these are essentially calculated from membrane potential gradients. However, there is no consensus as to what causes this regional RMP and slow wave heterogeneity and thus the resulting formulations presented here provide the desired effect but may or may not adequately represent the underlying cause.

Nonlinear cable models have been used to describe the electrophysiology of a variety of electrically excitable tissues including the intestinal musculature, neurons, cardiac muscle, and skeletal muscle. In the GI tract, a similar approach was employed by Edwards and Hirst to describe slow wave propagation through the antral wall (from serosa to mucosa). In this study, the longitudinal propagation of slow wave activity has been selected as a test system and this has been simulated using a coupled dual-cable model, running from the proximal fundus down to the pylorus along the greater curvature of the stomach.

**MATERIALS AND METHODS**

**Single-Cell Models**

Biophysically based quantitative descriptions of ICC and gastric smooth muscle single-cell electrophysiology have recently been developed. The models are based on Hodgkin–Huxley-like electrical circuit descriptions of the plasma membrane and are represented by a parallel plate capacitor connected in parallel with variable resistances representing the different ion channels. The interested reader is referred to the original model descriptions for further details of the model components, construction, and validation.

**ICC Entrainment**

While the mechanisms underlying this inherent chronotropicity are not well understood, it is known that IP3 has the ability to regulate slow wave frequency. In the absence of compelling physiological data on other potential mechanisms, here the intracellular IP3 concentration has been used as a proxy to control the intrinsic pacemaker frequency of the ICC. A linear IP3 concentration gradient was included in the

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**FIGURE 1.** Schematic of the revised ICC model including a representation of the mitochondria (Mito), endoplasmic reticulum (ER), and submembrane pacemaker space (PU). A fraction of the voltage-dependent dihydropyridine-resistant Ca\(^{2+}\) current (\(I_{VDDR}\)) has been added to the PU (\(I_{VDDR_{PU}}\)) to act as a voltage to Ca\(^{2+}\) transducer and thereby entrain intracellular Ca\(^{2+}\) cycling. Also in the plasma membrane of the pacemaker unit are non-specific cationic channels (\(I_{NSCC}\)) and a phenomenological Ca\(^{2+}\) extrusion mechanism (\(I_{CaEXT_{PU}}\)).
model with a concentration ranging from 645 nM at the top of the fundus to 600 nM at the pylorus, yielding an entrained pacing frequency of 3.1 cycles per minute (cpm). A higher IP3 concentration corresponds to a significant rate of internal Ca\textsuperscript{2+} cycling and although pacemaker function may depend mainly on Ca\textsuperscript{2+} and not voltage, the entrainment that occurs during slow wave propagation is likely to act through a voltage and not Ca\textsuperscript{2+}-dependent mechanism.\textsuperscript{27}

In many excitable tissues, e.g., cardiac muscle, the entrainment of cells distal to the primary pacemaker or stimulus occurs through a voltage-dependent coupling mechanism. Slow wave propagation velocities are significantly faster than can be explained by Ca\textsuperscript{2+} waves and although pacemaker function may depend mainly on Ca\textsuperscript{2+} and not voltage, the entrainment that occurs during slow wave propagation is likely to act through a voltage and not Ca\textsuperscript{2+}-dependent mechanism.\textsuperscript{27} However, the non-specific cation conductance that forms the initial electrical event in the single-cell ICC model is calcium inhibited and voltage independent, meaning that unlike other cells, the formation of the upstroke is largely voltage insensitive and thus does not exhibit voltage threshold behavior.\textsuperscript{27} As demonstrated later in Fig. 3 where the interaction of two coupled ICC are examined, simple voltage-based coupling, as is common in other cell systems, appears to be insufficient in this case with a voltage stimulus being unable to provide stable entrainment. To allow the intrinsic frequency of slow wave generation in an ICC to be entrained, the addition of a transducer that converts a voltage signal from the slow wave to a calcium signal in the ICC pacemaker unit is proposed. Recent experimental evidence has shown that Ca\textsuperscript{2+} entry through a voltage dependent, dihydropyridine-resistant pathway is involved in the coordination of slow wave activity.\textsuperscript{2,23} Therefore, the proposed entrainment mechanism was formulated by directing a small percentage of the whole cell voltage dependent \textit{I}_{VDDR} conductance into the aggregate ICC pacemaker submembrane space as shown in Fig. 1.

The Ca\textsuperscript{2+} concentration in this space was therefore calculated using

\[ \frac{d[Ca^{2+}]_{PU}}{dt} = (I_{NaCa} - I_{Uni}) \frac{V_{mito}}{V_{PU}} + (I_{ERout} - I_{SERCA}) \frac{V_{ER}}{V_{PU}} - I_{leak} \ \frac{V_{cyt}}{V_{PU}} - \left[ I_{VDDRPU} + I_{CaEXTPU} \right] \]

where \( I_{NaCa} \) and \( I_{Uni} \) represent mitochondrial Ca\textsuperscript{2+} fluxes, \( I_{ERout} \) and \( I_{SERCA} \) represent Ca\textsuperscript{2+} fluxes from the endoplasmic reticulum, \( I_{leak} \) is a leakage flux between the pacemaker region and the cytosol, \( z \) is valence, and \( F \) is Faraday’s constant. The \( V_{mito}, V_{ER}, V_{cyt}, \) and \( V_{PU} \) represent the volume fractions for the mitochondria, endoplasmic reticulum, cytosol, and pacemaker submembrane space, respectively. Each of these terms were calculated following Corrias and Buist.\textsuperscript{7} The \( I_{VDDRPU} \) in the pacemaker unit coexists with \( I_{VDDR} \) in the cell and has the same gating dynamics. \( I_{VDDRPU} \) has a maximum conductance of \( G_{VDDR}d_{PU} \) and consequently \( I_{VDDR} \) has a maximum conductance of \( G_{VDDR}(1 - d_{PU}) \). The Nernst potential for \( I_{VDDRPU} \) was based on the pacemaker calcium concentration, whereas \( I_{VDDR} \) used the intracellular calcium concentration. \( I_{VDDRPU} \) was therefore formulated as

\[ I_{VDDRPU} = G_{VDDR}d_{PU}I_{VDDR}f_{VDDR}(V_{m} - E_{Capu}) \]

where \( G_{VDDR}, d_{VDDR}, \) and \( f_{VDDR} \) are the maximum channel conductance, activation gate, and inactivation gate, respectively, from Corrias and Buist.\textsuperscript{7} \( d_{PU} \) is the fraction of the \( I_{VDDR} \) conductance and was set to 0.04, while \( E_{Capu} \) was calculated using the Nernst equation. Thus, when the membrane potential of an ICC is increased due to the depolarization of its neighbor or through an external stimulus, this mechanism provides an injection of Ca\textsuperscript{2+} into the pacemaker space and as such is able to entrain the internal rate of Ca\textsuperscript{2+} cycling. As the primary means of removing Ca\textsuperscript{2+} from the pacemaker space has yet to be determined, a phenomenological model of Ca\textsuperscript{2+} extrusion from the pacemaker space, \( I_{CaEXTPU} \), was adopted to allow long-term homeostasis to be maintained,

\[ I_{CaEXTPU} = \left( \frac{I_{Capu_{max}}}{1 + \exp \left[ \frac{([Ca^{2+}]_{PU} - C_{850})}{k} \right]} \right) \]

Here \( I_{Capu_{max}} \) was set to be 0.000315 mM/ms, whereas \( C_{850} \) and \( k \) were set to be 100 nM and 15, respectively.

**RMP Gradient**

Experimental evidence suggests that GI RMP gradients may result from the regulation of outward K+ currents,\textsuperscript{11,12} although a role for inward currents in the regulation of the RMP cannot be ruled out. K+ channels are known to be regulated by a large number of bioactive chemicals including gaseous mediators, neurotransmitters, prostaglandins, and inflammatory mediators.\textsuperscript{5,11} Insufficient evidence exists to properly quantify the role of each of these factors in determining the RMP but, on the other hand, in order to generate realistic tissue level behavior a RMP gradient is needed. The approach adopted here follows that proposed by Farrugia et al.\textsuperscript{12} whereby carbon monoxide (CO) plays a key role in the regulation of K+ channels in the GI tract and may therefore be one of the primary
factors that controls the RMP. Although this role is assigned solely to CO here, it is likely that a combination of factors is responsible for the observed RMP gradients.

Carbon monoxide is produced endogenously in many human tissues (see Durante et al.9 for a review). Recent experimental evidence has suggested that ICC in the GI tract, in particular the ICC-MY, produce CO through a heme-oxygenase-2 (HO-2)-mediated pathway.18,31 Experimental observations from genetically modified HO-2-null mice showing a lack of a membrane potential gradient12 appear to suggest a relationship between the RMP and CO concentration gradients. However, the mechanism through which CO causes membrane hyperpolarization in GI SMCs is yet to be fully elucidated.

Here it was assumed that CO is primarily produced by ICC-MY and therefore the distribution of CO production was chosen to follow the relative density of ICC-MY, which are largely absent in the fundus but present in the corpus and antrum. Given a normalized coordinate, $\xi$, ranging from zero to one along the length of the stomach, the relative ICC-MY density was described by

$$n_{\text{ICC}} = n(2\xi + (1 - z)/(1 + \exp[-(\xi - \xi_{50})/k])$$

(4)

where $n$, the density of SMC relative to each pacemaker ICC, was set to be 100, $z$ was 0.005, $\xi_{50}$ was 0.5, and $k$ was 0.01. The corresponding distribution of CO production was described by

$$[\text{CO}] = 0.1 + 0.4/(1 + \exp[-(\xi - \xi_{50})/k])$$

(5)

where the production levels were based on experimental measurements from the canine stomach showing that the CO concentration increases from 0.1 nmol/mg of wet tissue in the fundus to around 0.5 nmol/mg of wet tissue in the antrum.12 These parameter values were specifically selected in order to reproduce the membrane potential recordings of Szurszewski,10 CO was assumed to affect the voltage-dependent and calcium-activated K+ channels of the adjacent SMC in a dose-dependent manner. The effect of CO on these channels was included through the introduction of a CO-dependent factor, $J_{\text{CO}} = 2.475[\text{CO}] - 0.2375$ as an additional gating variable for the voltage-dependent K+ currents. The coefficients were chosen in order to replicate the RMP gradients described in the canine stomach.30

### Continuum model

A non-linear dual-cable model, similar to that described by Aliev et al.,1 was adopted for quantifying the longitudinal gradients in intrinsic pacemaker frequency and RMP present in the stomach. The spatial

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
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<tbody>
<tr>
<td>$\sigma_{\text{ICC}}$</td>
<td>0.3 mS/mm</td>
</tr>
<tr>
<td>$\sigma_{\text{SMC}}$</td>
<td>0.2 mS/mm</td>
</tr>
<tr>
<td>$A_{\text{ICC}}$</td>
<td>100 mm$^{-1}$</td>
</tr>
<tr>
<td>$A_{\text{SMC}}$</td>
<td>100 mm$^{-1}$</td>
</tr>
<tr>
<td>$C_{\text{mICC}}$</td>
<td>0.01 $\mu$F/mm$^2$</td>
</tr>
<tr>
<td>$C_{\text{mSMC}}$</td>
<td>0.01 $\mu$F/mm$^2$</td>
</tr>
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</table>

distribution of ICC and SMC were each described by a cable model as described in Eqs. (6) and (7).

$$\sigma_{\text{ICC}} \frac{\partial^2 V_{\text{m(ICC)}}}{\partial x^2} = A_{\text{m(ICC)}} \times \left( C_{\text{mICC}} \frac{\partial V_{\text{m(ICC)}}}{\partial t} + (I_{\text{ion(ICC)}} - I_{\text{coup}}) \right)$$

(6)

$$\sigma_{\text{SMC}} \frac{\partial^2 V_{\text{m(SMC)}}}{\partial x^2} = A_{\text{m(SMC)}} \times \left( C_{\text{mSMC}} \frac{\partial V_{\text{m(SMC)}}}{\partial t} + (I_{\text{ion(SMC)}} + I_{\text{coup}}) \right)$$

(7)

Here, with the appropriate subscripts differentiating ICC and SMC parameters, $\sigma$ is the axial conductivity, $V_{\text{m}}$ is the membrane potential, $x$ is a spatial coordinate along the axis of the fiber, $A_{\text{m}}$ is the surface-to-volume ratio, $C_{\text{m}}$ is the membrane capacitance, and $I_{\text{ion}}$ represents the summation of all the ionic currents. The values selected for these parameters are given in Table 1.

At each point in space, the ICC and SMC were electrically coupled via a constant conductance of $g_{\text{coup}} = 0.005$ mS/mm$^2$, designed to represent the presence of gap junctions coupling the SMC to the ICC. The resulting current crosses the outer membrane of both cells and is thus treated like an additional ionic current.

$$I_{\text{coup}} = g_{\text{coup}}n_{\text{ICC}}(V_{\text{m(ICC)}} - V_{\text{m(SMC)}})$$

(8)

The distance from the proximal fundus to the pylorus was estimated based on images from the Visible Human Project following a path along the greater curvature.28 The result was a 337-mm long cable that was discretized at a 1-mm resolution. Equations (6) and (7) were solved using an implicit forward time central space finite difference approach with a time step of 0.1 ms, whereas the ionic currents were solved using an explicit fourth-order Runge–Kutta scheme.

### RESULTS

#### ICC Entrainment

In the absence of the link between a slow wave depolarization and calcium cycling in the pacemaker
subspace, entrainment was unsuccessful as the timing of the internal calcium cycling among the pacemaker subspace, the endoplasmic reticulum, and the mitochondria remained unchanged and consequently the timing of slow wave initiation also remained unchanged. Single-cell simulations were performed to test whether or not the entrainment mechanism described here allowed the ICC to be synchronized to an external stimulus of a higher frequency, as is the case when the antrum is entrained by the corpus. For this purpose, a periodic voltage stimulus with a frequency of 3.1 cpm was injected into an ICC with an intrinsic frequency of 2.7 cpm. The stimulus had a height of 10 mV and a pulse width of 200 ms. Figure 2 shows an ICC, initially generating slow waves at its intrinsic frequency, being successfully entrained by the higher frequency external stimulus.

Simulations of 2 h of slow wave activity demonstrated an unchanged intrinsic frequency in the absence of an external stimulus, and the maintenance of entrainment when the stimulus was present (data not shown). Equivalent simulations were also performed to ensure that two ICC with different intrinsic frequencies could successfully entrain each other (data not shown).

It can be seen from Fig. 2 that the entrainment of the ICC is not instantaneous. One possible explanation for this is the refractory period of the pacemaker apparatus. After examining a number of different scenarios, it appears that the entrainment engages when the stimulus occurs close to the expected onset of a slow wave. If the first stimulus is given at such a time then the entrainment is immediate. In Fig. 2 this occurs after approximately 140 s, but if the first stimulus is delayed by 0.5 s then the second and subsequent slow waves are entrained.

In Fig. 3, two ICC are connected to each other via Eq. (8) (with $n_{ICC}=1$) to illustrate the need for the proposed entrainment mechanism. In the top panel of Fig. 3, two uncoupled cells can be seen generating spontaneous slow waves at different frequencies, 3.2 and 3.0 cpm. Should the cells be coupled via Eq. (8) in the absence of the proposed voltage-to-calcium pacemaker transduction mechanism the result is as shown in the middle panel of Fig. 3. This erratic result occurred regardless of the duration of the simulation and the initial phase difference of the two cells, and did not resolve itself after several hours of simulation time. The equivalent result when the proposed entrainment

![FIGURE 2](image1.png)

**FIGURE 2.** Single ICC being entrained by an external stimulus. The dark bars at the bottom of the figure show the timing of the stimulus and the light line shows the ICC membrane potential over time. The stimulus was applied at a frequency of 3.1 cpm to a cell whose intrinsic slow wave frequency was 2.7 cpm. Entrainment occurs at approximately 140 s and is stable for 1 h of simulation time (data not shown).

![FIGURE 3](image2.png)

**FIGURE 3.** Two ICC pacing at different frequencies. Horizontal axes are time in seconds and vertical axes are ICC membrane potential in mV. The top panel shows two uncoupled ICC, one pacing at 3.2 cpm (light solid line) and the other at 3.0 cpm (dark dashed line). If the two cells are directly coupled via voltage in the absence of the entrainment mechanism described here the result is disordered behavior as shown in the middle panel. With the adoption of the proposed entrainment mechanism the two ICC are successfully coupled as shown in the lower panel at a frequency slightly lower than that of the faster pacing cell.
mechanism was adopted is displayed in the lower panel of Fig. 3 where stable entrainment can be seen.

**RMP Gradient and Slow Wave Propagation**

Equation (4) describes a relatively sharp transition between the absence of ICC-MY in the fundus and the presence of ICC-MY in the corpus and antrum, and an equally sharp transition in CO production is described by Eq. (5). However, the resulting RMP gradient is significantly less sharp and the degree of smoothing is directly related to the tissue conductivities in that higher conductivities result in a smoother transition. Figure 4 shows a spatiotemporal plot of the SMC membrane potential distribution along the stomach (horizontal axis) over time (vertical axis), and below it a cross-sectional view of these data showing the membrane potential range for all time as a function of distance along the stomach. Here, it can be clearly seen that the fundus undergoes relatively small membrane potential changes relative to the antrum due to the reduced RMP polarization in the fundus.

In the physiologically coupled situation, the RMP in the SMC varied from $-42.2$ mV at the top of the fundus to around $-60$ mV in the corpus and decreased further to $-68$ mV in the antrum. This is consistent with experimental observations in which the RMP was found to be $-40$ mV in guinea pig gastric fundus 16 and $-48$ mV in canine gastric fundus. 30 In the canine mid orad corpus the RMP was found to be around $-60$ mV and was $-69$ mV in the orad antrum. 30

As Eq. (4) includes a low density of pacing ICC in the fundus, slow waves are initiated in the proximal stomach but are of small amplitude (1–3 mV) until they reach the orad corpus where the decrease in the RMP and a modest increase in the peak potential sees the slow wave amplitude transiently increase in the corpus through to a maximum of 27 mV in the distal antrum, a value slightly smaller than the 30 mV recorded from guinea pig antrum by Edwards and Hirst.10 Extracting temporal traces at different location along the stomach (fundus, very orad corpus, mid orad corpus, and antrum) further illustrates both the propagation of slow waves down the stomach and the increase in slow wave amplitude as shown in Fig. 5. If required, slow wave activity in the fundus can be readily eliminated by removing the ICC-MY from that region. In this situation slow waves were initiated in the mid orad corpus.

**DISCUSSION**

Chronotropic mechanisms in the ICC network have been the object of increasing interest over the past few years. 27 When studied in isolation, ICC from different
regions of the stomach pace at different frequencies. The existence of this frequency gradient along the longitudinal axis of the stomach has been shown in both dogs and guinea pigs. In the intact stomach, on the other hand, there appears to be one dominant frequency suggesting the existence of an entrainment mechanism. Such a system is not without precedent and it is widely known that the sinoatrial node cells in the heart pace faster than, for example, the atrioventricular node cells but in a healthy heart there is only one heart rate. In the heart voltage coupling alone appears to be sufficient whereby distal cells are activated via currents flowing through gap junctions. Here, however, as illustrated in Fig. 3, voltage coupling alone was able to depolarize the cell membrane as expected but was unable to entrain the internal Ca$^{2+}$ cycling that sets the intrinsic pacing frequency of an ICC.

Recent experimental evidence suggests that slow wave propagation, and hence ICC entrainment, may depend on Ca$^{2+}$ entry through voltage-dependent DHP-resistant pathways. It has been shown that slow wave propagation in antral muscle strips was blocked when Ca$^{2+}$ ions were removed from the extracellular solution and in the presence of inhibitors of DHP-resistant Ca$^{2+}$ channels. In line with this evidence, 4% of the $I_{VDDR}$ current was redirected into the submembrane pacemaking space to provide the link between membrane depolarization and pacing frequency, remembering that the non-specific cation channels are not voltage sensitive, and upon implementation successful entrainment was achieved. The value of 4% was the smallest proportion of $I_{VDDR}$ that could consistently entrain the ICC over the physiological range of pacing frequencies.

Interestingly, another possible pathway was already present within the existing ICC description through $I_{leak}$, the Ca$^{2+}$ leakage between the pacemaker space and the cytosol. A significant proportion of cellular depolarization in ICC can be attributed to the influx of Ca$^{2+}$ ions as ICC contain only relatively small numbers of Na$^+$ channels. Therefore, one might expect that upon depolarization, Ca$^{2+}$ ions may enter the cell and subsequently influence the pacemaker mechanism via $I_{leak}$. Here, however, altering the amplitude of $I_{leak}$ (by altering its conductance) did not result in stable entrainment. This is possibly due to a timing issue. Successful entrainment was achieved in Fig. 2 when the stimulus pulse and the slow wave upstroke occurred at approximately the same time, whereas there is a delay between the upstroke and Ca$^{2+}$ entering the pacemaker space via $I_{leak}$.

The existence of an intrinsic frequency gradient of the pacing cells is essential if one wishes to simulate the propagation of the slow waves from one point of the stomach to another. Without such a frequency gradient, all cells would activate concurrently, giving rise to unrealistic simultaneous depolarizations. The intrinsic ICC frequency was therefore regulated by adjusting the value of the intracellular IP3 concentration. IP3 has been proposed as a regulator of slow wave frequency in the murine stomach and small intestine, although it remains unclear whether the existing frequency gradient in the stomach is achieved through IP3 or by other means. If the latter turns out to be the case, the cellular models can be readily modified to incorporate the newly found mechanism. As no direct recordings have been performed to ascertain the expected IP3 concentration in an ICC, the concentrations were selected based on their ability to produce slow waves of the desired frequency.

One question that arises is that of what factors affect the velocity of slow wave propagation. As is the case in the predicate work of Aliev et al., here the primary determinant of the slow wave conduction velocity appears to be the intrinsic pacing frequency of the ICC and this is moderated by the ICC and SMC tissue conductivities. The conduction velocity can therefore be altered by adjusting the IP3 profile along the cable, and to a lesser extent by altering $\sigma_{ICC}$ and/or $\sigma_{SMC}$. The imposition of the RMP does have a minor effect on the conduction velocity whereby the velocity is somewhat reduced in response to the smaller amplitude slow waves that are present where the RMP is less polarized.

Although there is experimental evidence to suggest a role for CO as a potential regulator of the gastric RMP through via a modulation of K$^+$ currents, care must be taken not to overstate this case. The mode of action of CO has not been fully elucidated and experimental evidence on different tissues has resulted in contradictory conclusions. Although most investigators appear to agree that a hyperpolarizing effect is obtained through an activation of K$^+$ currents by CO, it is not clear which particular K$^+$ currents are involved and how they are augmented. In vascular SMCs, a direct biochemical modification of Ca$^{2+}$-dependent K$^+$ channels ($K_{Ca}$) has been proposed. In guinea pig gastric SMCs, a cGMP- and PKG-dependent vortical intracellular mobilization of Ca$^{2+}$ ions has been suggested to cause the augmentation of the K$^+$ currents. In human intestinal myocytes, on the other hand, no change in intracellular Ca$^{2+}$ levels was noted after the application of exogenous CO, supporting previous suggestions that the augmentation of the K$^+$ currents appears to be due to voltage dependent and not Ca$^{2+}$-activated K$^+$ channels. Here it was assumed that CO affected voltage dependent and large conductance Ca$^{2+}$-activated K$^+$ channels (BK) were activated by CO as BK channels are also believed to exhibit voltage dependence.
Furthermore, the list of endogenous bioactive compounds that could potentially produce similar effects on the RMP given an appropriate spatial distribution includes the gaseous mediators nitric oxide (NO) and hydrogen sulfide (H₂S), adenosine triphosphate, vasoactive intestinal peptide, hormones, paracrine substances, and inflammatory mediators. However, in order to generate realistic tissue and organ level slow wave activity RMP gradients are required and thus a selection must be made as to how these should be described. The choice of a mechanism based on CO production by ICC-MY was made due to the availability of published supporting experimental data. Should alternative evidence be presented in the future then it is a simple matter to either add onto or replace the existing K⁺ regulation structure, but the results of such a change must still produce results consistent with the RMP gradients recorded from intact tissue preparations.

A significant issue that has yet to be resolved in this study is the lack of sufficient experimental data to properly parameterize the tissue (cable) model. In this study a parameter set has been chosen to reproduce the earlier work of Szurszewski³⁰ where traces of membrane potential over time were recorded from a small number of finite locations along the canine stomach. The model parameterization, therefore, is limited to this particular case and a re-parameterization is likely to be required should the reproduction of other instances be of interest. Several groups are starting to make higher resolution experimental recordings that may yield better data and hence a better parameterization. Recent extracellular recordings by Lammers et al.²² have the potential to greatly alter our understanding of slow wave generation in the proximal stomach. Large amplitude slow waves have been recorded from proximal regions that were previously thought to be quiescent. High-resolution extracellular recordings of porcine gastric electrical activity have also recently been obtained by Du et al.⁸ using both printed circuit board electrode arrays and an epoxy-embedded electrode array. Extracellular potentials can be obtained from a spatial transmembrane potential distribution by solving the extracellular bidomain equation if required (see, e.g., Pullan et al.²⁶). However, the problem with interpreting extracellular recordings directly is that the proximal high amplitude recordings of Lammers et al. could be caused by either a more polarized RMP or by higher amplitude slow waves imposed on a relatively depolarized RMP. Should they be caused by a more polarized RMP, as is the case in the antrum, then this may be easily represented by altering the CO profile along the stomach. However, if they are high amplitude electrical events taking place over a depolarized RMP then the composition of the cellular descriptions must be revisited. It may also be necessary to extend the current framework to include a description of the ICC-IM if it is shown that these cells are responsible for the observed early activations. Once these factors have been addressed, such high-resolution data sets will be an invaluable resource for parameterizing tissue models such as that presented here.

REFERENCES


