Setting the pace for GI motility: ryanodine receptors and IP\textsubscript{3} receptors within interstitial cells of Cajal. Focus on “Intracellular Ca\textsuperscript{2+} release from endoplasmic reticulum regulates slow wave currents and pacemaker activity of interstitial cells of Cajal”

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THE GENERATION OF SPONTANEOUS phasic contractions within the wall of the gastrointestinal (GI) tract drives segmentation and peristalsis, thereby enabling proper food digestion. These rhythmical contractions, an intrinsic property of the gut wall, involve a triad of electrically coupled cell types: smooth muscle, interstitial cells of Cajal (ICC), and recently identified PDGF receptor (PDGFR)-positive cells, referred to as the SIP syncytium (5). Of these three cell types, ICC are now known to trigger the rhythmicity of spontaneous phasic contractions in the GI tract as disruption of ICC development abolishes spontaneous GI motility (3, also see ref. 5 for a recent review). Once ICC were established as the GI pacemaker, research focus shifted to defining the cellular mechanisms underlying the rhythmical electrical activity of these cells.

ICC are fibroblast-like cells interspersed throughout the circular and longitudinal muscle layers of the gut wall and are electrically coupled to each other and to adjacent smooth muscle. ICC pacemaker activity, in the form of slow wave currents, results in rhythmic membrane potential depolarizations that spread to smooth muscle via gap junctions. This electrical signal triggers the opening of voltage-activated Ca\textsuperscript{2+} channels and excitation-contraction coupling, resulting in the characteristic phasic contractions of the GI wall (5). Hirst and Edwards (2) established that ICC low-amplitude spontaneous transient membrane potential depolarizations (STDs) lead to slow wave current generation (2). Sanders and colleagues later discovered that STDs were due to spontaneous transient inward currents (STICs) caused by anoctamin 1 (ANO1), Ca\textsuperscript{2+}-activated Cl\textsuperscript{−} channels highly expressed by ICC (8). Further, STDs/STICs increase the open-state probability of T-type voltage-dependent Ca\textsuperscript{2+} channels causing Ca\textsuperscript{2+} entry and the prolonged activation of additional ANO1 channels to generate slow wave currents (5). Importantly, these studies established ANO1 channel activity in the form of both STDs/STICs and slow waves as the basis of ICC pacemaker activity. However, the underlying events initiating STD/STIC activity remained unresolved.

In this issue of American Journal of Physiology-Cell Physiology, Zhu et al. (9) take a unique approach exploring the roles of ryanodine-sensitive Ca\textsuperscript{2+} release channels (ryanodine receptors or RyRs), IP\textsubscript{3}-sensitive Ca\textsuperscript{2+} release channels (IP\textsubscript{3} receptors or IP\textsubscript{3}Rs), and endoplasmic reticulum (ER) Ca\textsuperscript{2+} stores in the activation of ICC ANO1 channels. Here, the investigators measured ANO1 channel activity (STICs), membrane potential (STDs), and slow wave ANO1 currents in ICC isolated from the small intestine of Kit\textsuperscript{+/copGFP} mice. ICC...
uniquely express Kit, a receptor tyrosine kinase (3). Kit-positive cells from Kit+/copGFP mice constitutively express the bright fluorescent protein, copGFP, enabling the authors to identify and study individual ICC following enzymatic dispersion of cells from the small intestine. Consistent with previous findings, the amplitude and frequency of STICs and STDs, as well as the peak amplitude of slow wave currents, were significantly reduced following ER Ca\textsuperscript{2+} depletion or blockade of ER IP\textsubscript{3}Rs (4, 6, 7). Importantly, the authors found that ryanodine receptor inhibition also disrupted STICs, STDs, and slow wave currents. This latter finding is at odds with observations made in intact GI tissue (4) and highlights the potential for complex interactions within the SIP syncytium. The method used by Zhu et al. (9) to study freshly isolated ICC avoids potential off-target effects of pharmacological agents on other cell types (e.g., PDGFR-positive cells and smooth muscle) and indicates that pacemaker activity in ICC is driven by Ca\textsuperscript{2+} release through both IP\textsubscript{3} and ryanodine receptors.

Advancing one step further, the authors measured ANO1 channel activity following dialysis of ICC with pipette solutions containing elevated intracellular Ca\textsuperscript{2+}. Remarkably, dialyzing ICC with up to 1 μM free Ca\textsuperscript{2+} failed to elicit ANO1-mediated currents. In contrast, HEK-293 cells expressing ANO1 channels exhibited substantial ANO1 currents when dialyzed with just 100 nM free Ca\textsuperscript{2+}. The authors conclude that the inability of increased bulk cytosolic Ca\textsuperscript{2+} to modulate ANO1 currents in native ICC suggests that these cells have a unique cyto-architecture providing a protected microenvironment around the pacemaker unit (1), requires close apposition of the subcellular ER cisternae with the ANO1 and T-type voltage-dependent Ca\textsuperscript{2+} channels clustered in the overlying plasma membrane (Fig. 1).

The study by Zhu et al. (9) bridges a large gap in our understanding of the mechanism driving pacemaker activity in ICC. Their results clearly identify the ER as the source of Ca\textsuperscript{2+} driving the ANO1 channel-mediated currents which underlie ICC pacemaker events. Further, this study demonstrates that IP\textsubscript{3} and ryanodine receptors are both involved in releasing ER Ca\textsuperscript{2+} into a restricted microdomain to activate ANO1 channel (STIC) currents. Importantly, these findings stimulate many additional questions. For example, what cellular factors trigger the rhythmic opening of IP\textsubscript{3} and ryanodine receptors to activate plasmaemmal ANO1 channels? What factors (e.g., differing ratios of IP\textsubscript{3}Rs and RyRs, perhaps?) dictate the varying slow wave properties generated by ICC throughout the GI tract? What influence do smooth muscle and PDGFR-positive cells exert on ICC pacemaker activity? Further, what cytoskeletal scaffolding proteins are involved in establishing and maintaining functional Ca\textsuperscript{2+}-signaling microdomains in ICC? It will also be important to visualize the unitary Ca\textsuperscript{2+} release events that activate STICs in ICC. Clearly, Zhu et al. (9) provide valuable new insights into the events underlying ICC pacemaker activity, but they also open doors to future work that will continue to define the events that allow us to gain nourishment from the food we love.

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AUTHOR CONTRIBUTIONS

A.P. and G.C.W. prepared the figure; A.P. and G.C.W. drafted the manuscript; A.P. and G.C.W. edited and revised the manuscript; A.P. and G.C.W. approved the final version of the manuscript.

REFERENCES