Do we need to revise the role of interstitial cells of Cajal in gastrointestinal motility?

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AS ITS NAME SUGGESTS, the túnica muscularis of the gastrointestinal tract is dominated by smooth muscle cells, which perform all the mechanical work required for digestion, absorption, and waste removal. The muscle layers also contain several other cell types, which, despite representing a much smaller percentage of the total cellular content, also contribute to gastrointestinal motility by regulating smooth muscle contractions. In this group belong interstitial cells of Cajal (named after Santiago Ramón y Cajal and commonly referred to as ICC), which represent ~5% of cells within the muscular coat. ICC are mesenchymal cells that have been described throughout the gastrointestinal tract of all vertebrates studied to date (15). They can be distinguished from other cell types on the basis of their light microscopic and ultrastructural morphology (16), gene expression pattern, and surface markers (2). Until the discovery of Kit, a type III receptor tyrosine kinase, as a light microscopic marker for ICC (10), investigators could only speculate on the function of these cells on the basis of less specific histochemical staining techniques and electron microscopy and by relying on relatively crude approaches to separate them from the rest of the tissues for physiological analyses. The identification of the interaction between Kit and stem cell factor (SCF or Kitl), its natural ligand, as the most specific target for genetic and pharmacological manipulation of ICC also paved the way for further, more mechanistic investigations. The first part of the “post-Kit era” culminated in the concept that functions previously attributed solely to smooth muscle cells and the extrinsic and intrinsic innervation of the gut may be performed, mediated, or aided by ICC (16). These include the generation and propagation of electrical slow waves underlying rhythmic contractile activity in the phasic parts of the gastrointestinal tract and mediation of communication between the smooth muscle and the autonomic (systemic and enteric) nerves. Later a role in mechanoreception was added (4), and the notion that changes in ICC populations likely play a role in the pathogenesis of various diseases also emerged (19). An exponential rise in interest and studies followed, which further enriched and refined these concepts and broadened the horizon by looking beyond the gut in search of ICC-like cells to explain functions shared by tubular, smooth muscle-lined organs. From these studies emerged a more integrative and nuanced view of the physiology and pathophysiology of gastrointestinal motility and of the role of ICC therein (5, 6, 13, 16). However, significant gaps in our knowledge remain, and it could be argued that filling those gaps and devising more rational therapeutic strategies for disorders involving ICC will require critical reevaluation of the existing data and the development and application of novel concepts and methodology to gastrointestinal motility research (13, 16).

In this issue of American Journal of Physiology Gastrointestinal and Liver Physiology, Dr. Sushil Sarna takes a critical look at the evidence supporting various roles of ICC in gastrointestinal motor functions and concludes that besides setting the membrane potential of smooth muscle cells by releasing the inhibitory gaseous neurotransmitter carbon monoxide, ICC play little, if any, physiological role (17). This concept is based on earlier views of the control of gastrointestinal motility that only assigned major roles to the smooth muscle and the autonomic (systemic and enteric) nervous system. In this paradigm, smooth muscle cells would produce electrical slow waves, perform mechanical work, and serve as the only relevant recipient and source of information needed for enteric reflexes and motor patterns.

Is such a dramatic return to an old paradigm really justified? In his review, Dr. Sarna points out data in the literature that he uses as an argument to refute the current concepts on the roles of ICC in slow-wave generation, mediation of neuromuscular neurotransmission, and mechanoreception. Reexamining concepts from a new aspect is always important for furthering scientific research, and this provocative review will certainly force many in the research community to reassess the literature. It is ultimately up to the informed reader to decide about the proper course of action in response to the issues raised. Are the new data strong enough to justify shutting down efforts in a particular direction? Is the alternative concept presented compelling enough to replace the one in question, or, rather, should we consider the highlighted inconsistencies as unsolved problems requiring that we “raise our game” and employ innovative approaches to get answers? The purpose of this editorial is to jump-start this process by examining Dr. Sarna’s hypothesis and by discussing whether the right action in response to the raised issues is to abandon and ignore ICC and resuscitate old paradigms or, alternatively, to give serious consideration to the remaining inconsistencies and then attempt to resolve them by applying novel, state-of-the-art concepts and methods of the postgenomic era to gastrointestinal motility research.

Which Cell has the Clock for Timing the Slow Waves?

Manifestations of electrical slow waves can be simultaneously recorded from both smooth muscle cells and ICC (5, 8), but which of these two cell types times their periodicity? There is no controversy that ICC possess an electrical pacemaker mechanism that is robust and capable of producing large-amplitude oscillations even in isolation. However, according to the hypothesis advanced in Sarna’s review, this clock is not the physiological source of slow waves and is...
replaced by another oscillator, presumably located in smooth muscle cells, which produces smaller, less regular, and more erratic activity but can still be detected in the absence of pacemaker ICC. To make these oscillations compatible with the rhythmic, large-amplitude, robust activity detectable in normal tissues, his hypothesis requires another factor that “stabilizes” them such as carbon monoxide, known to be generated by ICC (12), which would accomplish this task by hyperpolarizing the primary pacemaker away from the slow-wave reversal potential. However, this hypothesis is problematic. The experimental data do not support the notion that changes in the resting membrane potential could account for the loss (or loss of detectability) of slow waves in tissues depleted of ICC. First, the quoted value for the slow-wave reversal potential (about −40 mV) is too low for the tissues in which the effects of ICC depletion were studied. For example, depolarization of normal murine gastric antrum tissues with carbachol to at least −30 mV still permitted the resolution of clear slow waves (7). Second, flat lines, sporadic slow waves, or erratic spiking activity have been recorded at resting membrane potentials considerably less negative than −40 mV both in murine gastric tissues pharmacologically depleted of ICC and in the small intestine of S/GFP (SCF mutant) mice and in the colon of Ws/Ws (Kit mutant) rats, which have profoundly reduced pacemaker ICC populations (1, 11, 14). A landmark study on the loss of slow waves in the small intestine of Wv/Wv (Kit mutant) mice reported an average resting membrane potential of −57.4 ± 1.8 mV (20). Moreover, mice with genomic deletion of heme oxygenase-2, the major synthetic enzyme for carbon monoxide in ICC, still exhibited regular slow waves (as depicted in Fig. 2 of the review). It is also interesting that, in many ICC-deficient tissues, these spikes are entrained by a pacemaker mechanism, which becomes more robust in response to distention (6). This pacemaker activity may originate from smooth muscle cells or residual ICC such as those that occur in the region of the deep muscular plexus (6) or, in larger animals, in intramuscular septa (8). However, no electrical oscillator mechanism has been described in smooth muscle cells. Perhaps, rather than abandoning ICC as pacemakers for electrical slow waves, a more productive approach would be to identify the source of this residual rhythmicity, describe the subcellular oscillator that drives it, and study its contribution to rhythmic contractions in health and disease, where it may gain particular importance. This task may require embracing novel technology such as selective harvesting of various cell types for large-scale molecular analyses, testing the hypotheses derived from them by gene knockout and knockdown studies, and, ultimately, by physiological and advanced imaging techniques at the whole-animal level.

On the Utility of Kit and SCF Mutant Rodents and the Natural History of Gastrointestinal Dysmotilities

The concepts that ICC mediate nitrergic inhibitory and cholinergic excitatory neuromuscular neurotransmission were largely based on experiments in gastric muscles of Wv/Wv mice and were not reexamined in the review. However, subsequent studies in other organs, in other mutant strains, or by utilizing different (e.g., in vivo) approaches have produced negative or at least less clear-cut results. Purinergic inhibition and noncholinergic (peptidergic) excitation are also relatively preserved in the congenital absence of intramuscular ICC (1, 21). These observations have prompted investigators to reconsider the concept that intramuscular ICC may be solely responsible for mediating neuromuscular neurotransmission and to acknowledge the possibility of direct parallel innervation of smooth muscle cells (1, 21). These issues were pointed out in the review, but do they justify the dismissal of a substantial body of evidence supporting a role for ICC and declaring that they cannot play a role in mediating neural inputs to the smooth muscle? To a great extent, arguments both in favor of and against the role of ICC were generated in mutant rodents, primarily Wv/Wv and S/GFP mice and Ws/Ws rats. Therefore, it is important to recognize not only the utility of these constitutive knockoutst but also the fact that, like any other model system, they are not without problems and potential confounding factors that must be carefully considered when interpreting experimental results (9). First, remote effects from a loss-of-function-type change (e.g., loss of ICC) may affect cells that express neither the affected gene nor another gene required for the action of the affected gene (e.g., Kit in the case of a SCF...
muscle in response to sodium nitroprusside and altered re-

cells, the primary target of these mutations. In fact, the evi-
dences proffered (lack of hyperpolarization of the smooth
muscle in response to sodium nitroprusside and altered re-
sponse to neostigmine in \( W/W^r \) fundus and antrum, respec-
tively) are better interpreted by the loss of intramuscular ICC
and their role in neuromuscular neurotransmission. Remote
effects can actually be useful in identifying unexpected func-
tions. The best example is the impaired development of vagal
intramuscular arrays in Kit and SCF mutant mice lacking
intramuscular ICC in the stomach, which not only provided the
first evidence for an involvement of ICC in afferent responses
to stretch, but also raised the possibility that ICC may be able
to release neurotrophic factors to attract developing axons (4).
Unfortunately, this line of evidence was not discussed in the
review. Another well-known problem with constitutive knock-
outs or mutants is that their phenotype may be affected by
developmental compensation (9). This may not only impact
electrical pacemaking but also neuromuscular neurotransmis-
sion in Kit/SCF mutant rodents. For example, in the absence of
intramuscular ICC, the sensitivity of the smooth muscle to
neurotransmitters may increase and lead to an overestimation
of the relative significance of direct innervation when ICC are
present. Indeed, a supersensitive phenotype has been demon-
strated at the molecular level in \( W/W^r \) mice (18). Assumption
of some ICC functions by other cells such as intramuscular
fibroblast-like cells may be another mechanism of develop-
mental compensation (3). The development of better, state-of-
the-art techniques for genetic manipulation (inducible knock-
outs and knockdown approaches targeting Kit, SCF, and other
relevant genes) (9) will help us address these issues, a likely
better strategy than simply dismissing the presently available
experimental evidence because of inconsistencies.

When interpreting the impact of a mutation on gastrointes-
tinal motor functions, we should also consider the fact that the
manifestations of many of the motility diseases are not nearly
as dramatic as in other organs, for example, certain major
cardiac arrhythmias. Many gastrointestinal dysmotilities do not
significantly affect survival or cause dramatic symptoms, and
their significance is in their impact on quality of life (13). It
follows that the effects of relevant mutations in rodents could
be easily missed without rigorous in vivo studies. For example,
daily food intake is not reduced in \( Sl/Spl \) mice, but meal size is
reduced and meal frequency increased (4), a pattern consistent
with impaired accommodation arising from a combined effect
of reduced neurotransmission and mechanoreception. Rela-
tively few studies have investigated in vivo gastrointestinal
functions in these rodents, and thus their gastrointestinal phe-
notype may yet hold surprises.

On the Value of Expecting the Unexpected

When considering a proposed function for a cell or other
entity, we frequently examine whether its features are consis-
tent with the perceived requirements of that particular function.
Such comparisons are important since they establish how well

the characteristics of the target of our investigations measure
up to an established reference or “idea” in the philosophical
sense. Although such idealistic arguments can and should be
used in determining function of particular cells such as ICC,
we also need to be careful to withdraw them when not sup-
ported by available experimental evidence. A good example is
the argument that ICC cannot actively propagate the electrical
oscillations underlying rhythmic contractions because the fre-
quency of the slow waves generated by their networks would
be phase locked over substantial areas of their networks, due to
strong coupling among ICC, and that this would not be com-
patible with nonpropagating (segmenting) contractions. The
experimental evidence argues against this idea. Propagation
distance and direction have been found to be variable in
myenteric ICC networks imaged in situ, and the activation of
the underlying smooth muscle bundles was also variable (8).
These findings are, in fact, compatible with the proposed
function. But even when an idealistic argument seems valid, it
should not detract from looking for answers beyond what is
available as evidence at a given time. For example, as Sarna
points out, the morphology of the myenteric ICC networks
certainly seems incompatible with the anisotropic nature of
slow-wave propagation. However, it has been shown that the
answer to this problem can be found outside the realm of the
primary pacemakers. In an elegant set of experiments, Hirst
and Edwards (5) demonstrated that the rapid circumferential
propagation of gastric slow waves is facilitated by the intra-
muscular ICC running parallel to the smooth muscle cells. It is
approaches such as these that advance, explore, and rigorously
test new ideas that will be needed to solve the logistical
problem of how intramuscular ICC could mediate the effects of
neural input to a sufficiently large number of smooth muscle
cells. Since ICC are already suspected to have a secretory
phenotype (16), could they fulfill the role of an integrator
receiving excitatory and inhibitory inputs and translating them
for smooth muscle cells by releasing mediators into the inter-
stitium (i.e., by volume transmission), integrating past concepts
with current evidence?

In summary, a growing body of evidence suggests that
normal gastrointestinal motility depends on interactions among
several cell types occurring within the smooth muscle layers.
The bulk of evidence suggests that ICC, just like other regu-
laratory cell types, perform specialized functions and should
retain their place among the key players. Rather than revering
to concepts that were developed before we had tools to study
ICC, a likely more fruitful approach is to incorporate our
present knowledge into an integrated model of gastrointestinal
motility. Unsolved issues remain and will undoubtedly con-
tinue to arise from such an integration of complex knowledge.
Finding solutions to these problems will likely require looking
beyond currently accepted models and approaches and applying
novel, state-of-the-art concepts and methods of the post-
genomic era to gastrointestinal motility research. New data will
certainly force us to continuously revise and refine the roles of
ICC but, most likely, not to ignore them.

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REFERENCES


