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Furthering the debate on the role of interstitial cells of Cajal in enteric inhibitory neuromuscular neurotransmission

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Chaudhury A. Furthering the debate on the role of interstitial cells of Cajal in enteric inhibitory neuromuscular neurotransmission. *Am J Physiol Cell Physiol* 311: C479–C481, 2016. First published July 6, 2016; doi:10.1152/ajpcell.00067.2016.—The gut, a muscular organ, performs a critical role in transporting ingested contents, yet it is also controlled to periodically stop transport to maximize digestion and toxin detection. The complex intraluminal composition and rheology challenge the mechanistic requirements of inhibitory neuromuscular neurotransmission. The interstitial cells of Cajal (ICCs)-generated slow wave may tune the promiscuous luminal chemical environment, which prepares the smooth muscle membrane potential for a depolarizing or hyperpolarizing response as needed. Slow waves are abolished during stimulation-induced inhibitory junction potentials (IJPs) due to purinergic-nitrergic tandem neurotransmission. Recent data demonstrating intact IJPs in a genomic knockout of ICCs provide rigorous evidence of the noncontribution of ICCs during evoked neurotransmission. This perspective article discusses the priority areas of investigations in enteric musculomotor transmission, for understanding its near-perfect design for chemical space sensing, as well as diseases in which the luminal transport braking process becomes dysfunctional, leading to delayed gastric emptying or intestinal transit.

ICC; interstitial cells of Cajal; neurotransmission; nitrergic; nitric oxide

THE IMPASSE IN DISCUSSIONS on the involvement of interstitial cells of Cajal (ICCs) in enteric neuromuscular transmission continues (12, 27). Two recent significant CrossTalk articles in the *Journal of Physiology* highlight the ongoing debate between supporters of the opposing theories. However, there are additional considerations to this debate, as outlined below.

First, we need to define the impact of neuronal release on slow waves. The slow wave, the signature electrophysiological responses emanating from ICCs, is due to highly coordinated cyclical ionic responses (15). These have been reported to be TTX sensitive (26), though the precise contributions of neural inputs have been incompletely delineated. I had earlier suggested that using novel models like Munc18-1 knockout may be a step forward in obtaining this critical information (9a). Because of exocytosis defects, vesicular neurotransmission is severely impeded in these mice. The variations of slow wave and inhibitory junction potentials (IJPs), if any, shall provide insights into the cellular components involved during the sequence of events from neural release to final smooth muscle motor response.

Second, multiple recordings have shown that when IJPs occur, slow waves are abolished (10, 19, 25, 26), suggesting that slow waves are desynchronized during evoked postjunctional neurophysiologic responses (9a). Recent experiments have provided strong evidence that fast and slow IJPs are clearly recordable in a genomic knockout model of ICCs (21). The straightforward inference from this novel mouse model is that ICCs do not play any role in genesis of IJPs (9a). These authors have, however, repeatedly ignored their landmark findings.

Given the high diffusibility of NO as 3,300 $\mu m^2/s$ (3, 23) and the latency between the electrical field stimulus (EFS) and occurrence of slow IJPs as ~100 ms, NO can travel a diameter of 3.3 μm^2 post-EFS, which is greater than the separation distance between the nerve terminal and the smooth muscle. It may be noted that these calculations are somewhat of an oversimplification; these would be more nonlinear, dependent on regional factors that keep binding NO upon release (and thus degrading free NO). ICCs possess a plethora of cellular machinery to interact with NO and will respond to released NO. However, it is unlikely that ICCs act as a complete sink for NO (3, 9a). The smooth nature of the IJP tracings (2) potentially suggests its genesis in the same cell. In fact, the authors have earlier suggested the repolarization mechanisms of the IJP as emanating from the smooth muscle cell (1). The summative effects of a purinergic response through a PDGFRα+ telocyte, a nitrergic response through ICCs, and then transmission through gap junctions, may make a smooth trace as well. However, because of the time domains and the necessity for differential spatial summation, this probability is unlikely. There is no obvious direct evidence to this hypothesis. I have reported the role of prejunctional cytoskeletal force-generating proteins like myosin Va in the tandem neural release of the vesicular and nonvesicular neurotransmitters (ATP and NO, respectively) (4, 7, 8), which may explain the coordinative response of fast and slow IJPs on a scale of a few seconds.

The authors have backed off from their initial suggestions that ICCs are key cellular components in transducing inhibitory neurotransmitter signals (21) and now made the alternate suggestions that ICCs act as initial cellular components to transduce the nitrergic inhibitory signal (24). The authors have named this phase of IJP as SIIa phase of slow IJP, followed by SIIb phase, which the authors hypothesize occurs in the smooth muscle (24). Interestingly, this SIIa phase (24) persisted in the IJP recorded from the genomic knockout model of ICCs (21). The authors fail to explain the rationale of persistence of IJP in ICC-GCKO (24). This strongly suggests either an "optional" role (12) or actually insignificant role for ICCs in evoked nitrergic signal transduction (3, 9a). The hyperpolarizations persisted after use of MRS2500 in both the ICC and smooth muscle-specific GCKO (24), again reiterating my earlier suggestions that the knockout models only inefficiently inhibit the target protein guanylyl cyclase (3). The authors have never tackled their divergent observations (21) from the newly pro-

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posed, potentially erroneous model of IJP (24). These confusing and inconclusive inferences merit careful validation, pending which assertions (27) should preferably be guarded. Knockout of soluble guanylyl cyclase produces not only vascular relaxation defects, but also gastrointestinal phenotypes. For example, complete deficiency of $\alpha_1\beta_1$ guanylyl cyclase has been demonstrated in three unrelated human families with coexistent vascular Moyamoya disease and achalasia (14).

The SIP syncytium (27), though a comfortable model for understanding the ill-defined transcellular diffusion of the chemico-electrical signal, merits rigorous clarification. For example, a diverse phenotype, with some cells c-kit+ and others PDGFRα+, is a challenge from the development perspective. Simple experiments like whether PDGFRα+ cells stain for c-kit can provide significant insights. Recent reports have provided preliminary observations that c-kit-PDGFRα co-stain in ICCs (13). Importantly, c-kit and PDGFRα both belong to type III tyrosine kinase receptors and are tandemly located on their genes (29). So it is not surprising that interstitial cells may stain for c-kit or PDGFRα and may not actually be diverse cell lineages.

The nature of the in vivo characteristics of the evoked stimuli and the summative contributions of the different components in the neuropil remains virtually unknown. The slow wave may be needed to tune for the promiscuous luminal chemical environment, which in turn trains the smooth muscle membrane potential for a depolarizing or hyperpolarizing response pro re nata. However, during nonbasal, evoked neurotransmission, the ICC-mediated responses may no longer be required, as the mechanical response is directly generated by the smooth muscle. The characteristics of the impulses traveling to the junctional endings of descending inhibitory innervation are not known. A stimulus of a single pulse of 1 Hz produces IJP (30), so it is likely that submaximal stimuli that produce slow waves are weaker frequencies. However, there surely must be huge synchrony in release of excitatory and inhibitory neurotransmitters from the junctional endings, which produces the alternate directions of the basal polarization waves and the range of smooth muscle membrane potentials. There is again no direct evidence for occurrence and synchrony of excitatory and inhibitory responses. However, observations from electrical and mechanical recordings are suggestive of the synchrony. For example, the occurrence of the on-contractions and off-contractions, and their occurrence during mechanical relaxation, hints towards temporal relationship between these phenomena.

The stimulus-induced neural release potentially should affect every structure in its vicinity: interstitial cells including ICCs, blood vessels, mast cells, macrophages, components of the extracellular matrix, and other mobile cellular elements like intraepithelial lymphocytes (IELs). These diverse cells have receptor repertoire for purines and nitric oxide (11, 20). The released chemicals from the nerve terminals shall affect all cells in its vicinity, which has an overall impact on the IJP and mechanical motor response. The slow IJP likely acts as a brake to the repolarization of the fast IJP to the resting membrane potential; because of its dependence on de novo release of gaseous nitric oxide, its duration is variable. The downstream effects of guanylyl cyclase activation in genesis of inhibitory potentials may be different in ICCs and smooth muscles, leading to differential regulation of genesis of the hyperpolarization sequence of the slow wave and the slow IJP in smooth muscles. For example, the type of chloride channel is different in ICCs and gastrointestinal smooth muscles. While there are extensive preliminary reports of anoctamin in ICCs (17), these chloride channels are absent in GI smooth muscles (17). In fact, GI smooth muscles may have completely different repertoire of chloride channels like CLC3 (personal observation).

We should have the resources to go to the bench to more accurately define the contributory roles of the different cellular components in enteric neuromuscular neurotransmission and its regulation, remembering the broad picture of the chief functions of the gut in nutrition acquisition and toxin/microbe detection. The gut can only perform its necessary role in moving luminal contents as a hollow muscular organ, periodically stopping this movement to maximize the digestive process and nutrient extraction, as well as to detect toxic components. The gut wall replaced by the ICCs does not function, as noted from long-segment diffuse ICC hyperplasia and neurofibromatosis (18).

In one of the CrossTalk articles, it is suggested that ramping up nitrergic transduction in ICCs may be a therapeutic option (28). However, caution needs to be exercised while making such empiric suggestions. I have earlier highlighted the challenge of bioelectronics in development of gastrointestinal motility therapeutics (5). Innovative approaches in theranostics are required (6). Careful reexamination of observations shall provide the next steps for understanding pathophysiology. For example, there is loss of ICCs in diabetes mellitus, but after a period, these cells may grow back (22). Given the intrinsic ability of ICCs to respond to stem cell factor, multicellular apoptosis in the gut wall in diabetes (9, 22), as well as regenerative responses (22), merits aggressive examination. Arguments should rise above semantic reconciliation and be directed at dissecting pathophysiology of gastrointestinal motility disorders to expand the currently limited therapeutic options.

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AUTHOR CONTRIBUTIONS

A.C. drafted manuscript; A.C. edited and revised manuscript; A.C. approved final version of manuscript.

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