

Chapter 2

A Personal Perspective on the Development of Our Understanding of the Myogenic Control Mechanisms of Gut Motor Function

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Myogenic control mechanisms play a role in all motor activities of the gut. Myogenic control systems are defined here as control systems that are intrinsic to the smooth muscle cells and/or interstitial cells of Cajal (ICC) and that can operate without an essential contribution of the intrinsic (ENS) and extrinsic nervous systems. In vivo however, the ENS and the myogenic control systems always work in cooperation. Although myogenic control plays a role in every gut organ, this review focuses on the peristaltic and segmentation activity of the small intestine. It provides some historical perspectives and some discussion on the development of our understanding of the cooperative nature of the myogenic and neurogenic control mechanisms. It highlights how some influential papers inadvertently provided hindrance to full understanding, it discusses how the guinea pig model has hampered acceptance of myogenic control systems and it provides some background into the genesis of our understanding of control mechanisms involving ICC.

The Dominance of the “Law of the Intestine”

Nothing has hampered the acceptance of myogenic control mechanisms more than the formulation of the “law of the intestine” by Bayliss and Starling. The likely reason is the elegant descriptions of the evidence for it and the attractive nature of such a simple and effective, easily understood mechanism. This despite the fact that Bayliss and Starling prominently described myogenic control systems as well. In Bayliss and Starling’s seminal paper from 1889 where the “law of the intestine”

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was formulated, the prominent existence of myogenic contractions was firmly established: “In the first place, we have the rhythmic pendular movements produced by simultaneous contractions of circular and longitudinal coats, and entirely myogenic in origin” (Bayliss and Starling 1899). These contractions were observed in the dog intestine occurring at 12/min, they were observed to propagate in both directions and have multiple origins along the intestine. The interesting thing is how the “law of the intestine” theory could have survived for so long as the stated dominant mechanism of peristalsis despite the concerns expressed from the early days. It was obvious already from Bayliss and Starlings’ work and confirmed by numerous authors in subsequent years that the law could often not be demonstrated in certain animal models or under certain conditions and that the weakest part was the inhibition of the musculature in front of the bolus, which was often not demonstrable. Nevertheless, the law survived and in the years that followed the notion of myogenic control somehow became less popular leading to exasperated statements from the Mayo Clinic gastroenterologist Alvarez. Alvarez wrote in 1922: “There are few statements in physiology upon which opinion is more unanimous than the one to the effect that the rhythmic contractions of the bowel are neurogenic, and due to impulses coming from Auerbach’s plexus. We find that statement in almost all of the textbooks and articles which we have consulted in the last eight years” (Alvarez 1922). Then he wrote: “Such unanimity is rather surprising, but it is still more surprising when we learn that practically all the research work done on the subject points clearly in the opposite direction”. This was followed by a discussion of the literature on this topic. At the end of his paper, Alvarez concluded: “... it should be pointed out that the question of the neurogenic or myogenic origin of the contractions is a more or less academic one because under normal conditions the muscle and nerve fibers are intimately associated and are designed to work together.” This sentiment was repeated in 2006 by Marcello Costa in his publication “All together now, from pacemakers to peristalsis” (Costa 2006). There has never been any doubt about the fact that the “law” can be demonstrated; the discussion should be to what extent are (most) normal peristaltic contractions governed by this law, by other neural programs and/or by myogenic controls systems. Statements on myogenic control have found their way in the literature at regular intervals, although neural control systems have always dominated the discussions on motor control. In 1986, Code and colleagues (Code et al. 1968) wrote: “the plexuses appear to program the motor action of the small bowel using the slow waves to consummate the program”.

The dominance of the ENS control of motility of the intestine is not without logic since we cannot survive without it (Huizinga et al. 2001) whereas the loss of the dominant pacemaker cells that generate the slow wave activity in animal models (Huizinga et al. 1995) does not lead to a dramatic failure of transit or absorption since alternative ways of propulsive contractile activity obviously develop.

The Incorporation of Interstitial Cells of Cajal in the Myogenic Control System

An important moment in the history of ICC physiology was the 9th International Symposium on Gastrointestinal Motility held in 1983 in Aix-en-Provence where Lars Thuneberg showed data on methylene blue mediated destruction of ICC-MP in the intestine and the loss of slow waves as a consequence. Thuneberg also showed video's of ICC in culture. For many of us, it was the first time that ICC came on our radar screen, and for many of us it was the start of incorporating ICC in our research protocols. But it took another 10 years to find convincing proof in the literature. Only a few months apart, two papers appeared, one at the end of 1994 and the other at the beginning of 1995 that provided evidence for and immediately firmly cemented a key role of interstitial cells of Cajal in the myogenic control system (Ward et al. 1994; Huizinga et al. 1995). It became clear that not smooth muscle cells but specialized pacemaker cells, the interstitial cells of Cajal were the generator of the omnipresent slow wave activity. This is how my laboratory got to write one of those papers: I have known Stephan (Steve) Collins for 30 years and in all these years of collaboration he has come to my office only once to show me a research paper he thought might be of interest to me, but once turned out to be enough. It was Maeda's paper describing how c-Kit antibodies could eliminate a certain cell type that would change the rhythmicity of intestinal contractions (Maeda et al. 1992). This was in 1993 and I can still see him standing in the doorway, casually giving me the paper he got from one of his post docs. I sat down at my desk and starting reading. After 1 h I could not contain my excitement and started walking up and down the hallway. Clearly this paper contained the gateway to proving or disproving the role of ICC in gut motility. I went back to Steve and told him that I wanted to work on this and how we should collaborate, but he told me that it was not his thing and I could go ahead. But the obstacles seemed large. The paper described how c-Kit antibodies could eliminate c-Kit positive cells from the intestine and at the same time affect the rhythmicity of the intestinal contractions. In the summer of 1993 I met Lars Thuneberg and Hanne Mikkelsen at the 14th International Symposium on Gastrointestinal Motility in Muskoka, Ontario and we discussed the project in Lars' hotel room and planned the immunohistochemistry and electron microscopy. It so happened that Mary Perdue occupied the office next to mine and she was working on WWv mice that lacked the c-Kit receptor (Perdue et al. 1991). She was interested in the role of mast cells in inflammation and I realized that the WWv mouse would be perfect for this study. But I knew little about the c-Kit receptor so I went to one of the experts, Bernstein at the University of Toronto. We decided to investigate the potential absence of ICC in the WWv mice using in situ hybridization. I remember one meeting at the University of Toronto. Thuneberg had come over to work with us on this project from Copenhagen. We wanted to show him the results

of the *in situ* hybridization that showed c-Kit positive ICC in control mice and no staining in WWv mice. When the figures were past on to him, he declared that we were mistaken, these brightly stained cells could not possibly be ICC because the size of the cells suggested them to be in an entirely different domain. Anxiety crept into the room and all eyes were on the calibration bars of the figures. Michael Kluppel, who had done the study, ran back to the lab to get the lab book. When the proper size of the cells was established, everything fell into place and we knew that we had proven the absence of ICC in the WWv mice. Lars went back to Copenhagen to confirm the *in situ* hybridization experiments with immunohistochemistry and in the mean time, John Malysz in my lab worked hard on getting electrical recordings from the control mice. It took many months to get reliable recording but John persisted and the result was one of the most exiting and convincing figures in the literature where we not only showed that WWv mice do not have slow wave activity but we also showed that the WWv intestine musculature is capable of generating action potentials indicating a normal musculature and the capability of contractile activity. Some time after we had started the experiments on the ICC in the mouse intestine I became convinced that Kent Sanders was probably experimenting with the same ideas since I discovered then that he had proof read the Maeda paper as mentioned in the acknowledgment section (Maeda et al. 1992). There was no doubt in my mind that Kent would have been just as excited about the paper as I was and that therefore he most definitely was working on the topic as well. I figured that the theory proven or disproven by two independent labs would be the best outcome. Just before we were ready to submit the paper to *Nature*, I met Casey VanBreen and told him about my adventures with ICC. He insisted that I should talk about this at a conference he was organizing and I agreed. Kent Sanders was the chairman of that particular session and both he and I presented our data on the ICC back to back. Seldom have I experienced more excited anticipation than waiting for the talk from Kent about his ICC research. Our talks were remarkably similar, also he had decided to work with WWv mice instead of injecting c-Kit antibody.

A few years later, the final piece of evidence was reported, again communicated through two papers published a few months apart (Koh et al. 1998; Thomsen et al. 1998). If ICC were truly pacemaker cells, they ought to exhibit spontaneous rhythmic inward currents when isolated, and smooth muscle cells ought not to have that property. And indeed, ICC isolated from the myenteric plexus area of the mouse small intestine produce spontaneous inward currents with a reversal potential of +10 mV (Thomsen et al. 1998) or +17 mV (Koh et al. 1998). The most likely candidate for the channels producing the inward current was thought to be a non-selective cation channel at the time (Koh et al. 1998; Thomsen et al. 1998). Now this hypothesis has been superseded in favour of chloride channels (Wright et al. 2012; Gomez-Pinilla et al. 2009; Hwang et al. 2009).

The Role of the Guinea Pig in Our Thinking About Control Mechanisms

There can be no doubt about the important role the guinea pig model has played in the development of our understanding of the role of the ENS in motor control. The data derived from this model has been phenomenal and forms at this moment the fundamental basis of our understanding of the ENS (Costa and Brookes 2008; Furness 2006). It so happens that the guinea pig intestine may not show slow wave activity when an electrode is penetrated into the musculature under certain conditions and this has led to the myogenic control system taking a back seat and in most studies on guinea pig motility it has not been discussed. It is interesting to note that the influential Trendelenburg method, so frequently used in guinea pig peristaltic research, started with a 1917 paper from Trendelenburg in which he showed that the peristaltic activity of the guinea pig could occur without any neural influence (Trendelenburg 2006). Although peristaltic contractions could be inhibited by blockers of neural activity, it was clear to Trendelenburg that “conduction of peristalsis in the small intestine can also, like the propagation of peristalsis in the stomach, proceed without a nervous ... conduction system”. Furthermore, he showed in numerous figures the extreme rhythmicity of the peristaltic activity that occurred at the frequency of the slow wave activity as later shown by others (Donnelly et al. 2001; Smith 1989). The fact is that the guinea pig intestine exhibits robust slow wave activity but this activity is not omnipresent, it develops in response to a stimulus such as distention (Donnelly et al. 2001). There is no doubt in my mind that the rhythmic peristaltic activity of the guinea pig small intestine, already shown by Trendelenburg to be able to occur without neural influence is governed by slow wave activity generated by interstitial cells of Cajal (Komuro and Zhou 1996) with the ENS being the major excitatory force for smooth muscle depolarization. The guinea pig intestine can switch from peristalsis to segmentation with segmentation still occurring at the slow wave frequency (Gwynne and Bornstein 2007). But the tendency to deny a role for myogenic activity in the generation of such segmentation activity is strong (Gwynne and Bornstein 2007).

Expanding the Role of ICC in Control of Motility: The Segmentation Motor Pattern

Almost all motor patterns in gut organs have as primary function the mixing of content. Although classical peristalsis is equated with propulsion, and this is certainly the case in the esophagus, the predominant effect in all other organs is mixing and exposing the content optimally to the mucosal surface, because the propulsion ends somewhere and the content is moving back; only very rarely does propulsion end with

evacuation of content from the body (Huizinga and Chen 2014). The segmentation motor pattern is different from peristalsis in that it contains only stationary or very short distance propagating contractions and hence is considered a specialized motor pattern for mixing and absorption. The segmentation motor pattern was described and illustrated by Cannon in 1902 based on X-ray observations and shown to be extremely rhythmic (Cannon 1902). Walter Alvarez was the first to find that the frequency of rhythmic segmenting contractions occurred at the frequency of a myogenic pacemaker and that in various regions of the intestine the frequency decreased in the same way as the pacemaker frequency decreased (Alvarez 1914). In 1968, Code and colleagues (Code et al. 1968) also recognized the role of slow waves in segmentation; the slow waves were thought to go in and out of excitable regions of smooth muscle fibers. Although it is clear that segmentation and intestinal pacemaker activity have been associated in many discussions, experimental evidence as to how pacemaker activity would *create* segmentation was lacking. And therefore the attention shifted to the enteric nervous system to find a neural program that would change peristalsis into segmentation. One hypothesis states that cholinergic motor neurons acting on muscarinic receptors periodically activate the musculature and that inhibitory neurons surround this contraction to finalize the motor pattern (Gwynne and Bornstein 2007; Gwynne et al. 2004). Another study states that CCK and 5-HT are critical for the generation of segmentation (Ellis et al. 2013). Segmentation has also been suggested to result from a reduced degree of synchrony of AH neuron activity and by sustained inhibition by the after hyperpolarization (Ferens et al. 2007; Huizinga and Chen 2014). A few years ago, my own hypothesis was that a contraction could activate enteric sensory neurons which would result in activation of short nitrergic inhibitory neurons to transiently inhibit a slow wave driven propulsive contraction so that it periodically became annihilated causing rhythmic segmentation. Until I saw a perfect segmentation motor pattern in the presence of TTX (Huizinga et al. 2014). While on sabbatical at the laboratory of Jihong Chen at Wuhan University we experimented with decanoic acid that had been shown to induce segmentation (Gwynne et al. 2004). We discovered a remarkable feature: decanoic acid changed the electrical activity of the intestine from regular slow waves to a waxing and waning pattern. At first we hypothesized that if two pacemaker sites from the ICC-MP network were sending slow wave activity into the musculature at slightly different frequencies, waxing and waning might occur and possibly segmentation (Fig. 2.1). This was based on ideas and evidence presented by Diamant, Bortoff and Suzuki (Diamant and Bortoff 1969; Suzuki et al. 1986). Indeed, when two sine waves of slightly different frequency interact by addition, a waxing and waning pattern develops. But in that case, the maximum amplitude becomes twice the original amplitude. When we confirmed the induction of waxing and waning by intracellular recording of the electrical activity, no change in maximum slow wave amplitude occurred (Pawelka and Huizinga 2015). Suzuki deduced the existence of two prominent similar frequencies in the waxing and waning pattern based on FFT analysis but FFT might not be the best method to evaluate frequencies in a signal that changes markedly over time. When we explored Continuous Wavelet Transform analysis, a single dominant slow wave frequency was obvious together with a low frequency

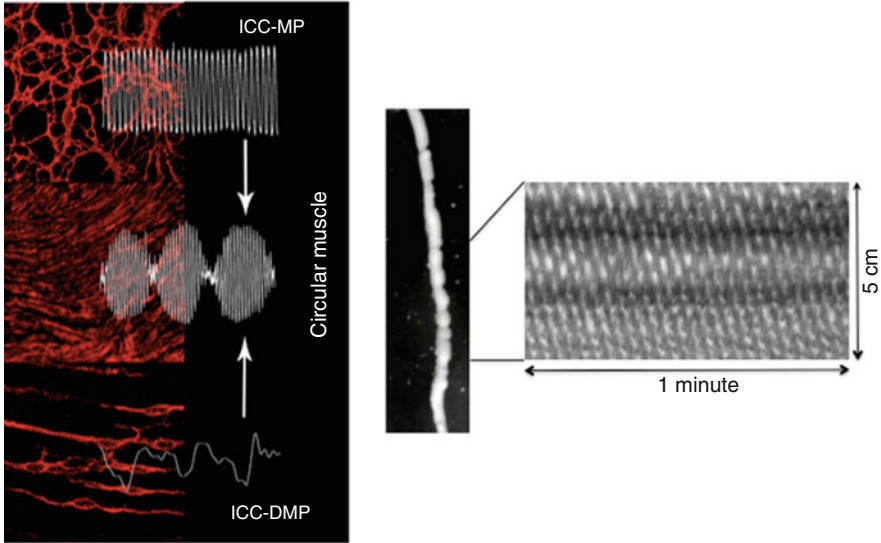


Fig. 2.1 ICC associated with the myenteric plexus (ICC-MP) generate slow wave activity. ICC associated with the deep muscular plexus (ICC-DMP) generate rhythmic transient depolarizations upon receiving specific stimuli such as decanoic acid or butyric acid, directly or indirectly. The two signals originating at opposite sides of the circular muscle layer can interact with each other by phase-amplitude coupling once propagated into the musculature. That is, the phase of the low frequency component modulates the amplitude of the high frequency component resulting in waxing and waning without increase in amplitude of the maximal slow wave. This electrical activity can orchestrate the checkered segmentation motor pattern. One frame of a video recording is shown showing multiple simultaneous circular muscle ring contractions, also evident in the spatio temporal map of the checkered segmentation motor pattern. Modified from Huizinga et al. (2014)

component. Calcium imaging gave us the hypothesis that the origin of the low frequency electrical signal was the network of ICC-DMP (Huizinga et al. 2014). Discussions with Bardakjian and McGinn at the University of Toronto led to an exploration of how the low and high frequency oscillations were interacting with each other within the musculature as coupled oscillators. Based on theories and methods of phase–amplitude coupling in the central nervous system (Tort et al. 2010) we obtained strong evidence that the waxing and waning electrical activity can be explained by the phase of the low frequency component modulating the amplitude of the high frequency component resulting in a waxing and waning motor pattern (Huizinga et al. 2014). Importantly, analysis of the checkered motor pattern of segmentation also provided evidence for phase–amplitude coupling underlying this motor pattern. The evidence that the pattern of segmentation can develop when a low and a high frequency myogenic pacemaker interact, does not exclude a role of the ENS in the development of segmentation *in vivo*. In most experimental conditions, the enteric nervous system will provide an essential stimulus for the motor activity to develop and hence a variety of nerve conduction blockers or neural receptor blockers will inhibit segmentation activity. Whether or not a motor pattern occurs in response

to nutrients or distention is often determined by the response of the ENS to the stimulus. This is also the case for segmentation, and several components of the ENS have been shown to be involved (Gwynne and Bornstein 2007). This neural activity then works in concert with the ICC pacemaker activities to generate the motor pattern of segmentation (Huizinga and Chen 2014).

In summary, myogenic control systems are present in the intestine and are part of the orchestration of most motor patterns. They provide the rhythmic propulsion within phase III of the migrating motor complexes (Hall et al. 1982) expressed during fasting at night, they provide rhythmicity and propulsion to peristaltic activity, in particular in the proximal intestine (Der-Silaphet et al. 1998), and they provide the basis for the segmentation motor pattern (Huizinga et al. 2014).

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