PERSPECTIVES

ANO1-ther brick in the wall – role of Ca^{2+}-activated Cl^- channels of interstitial cells of Cajal in cholinergic motor control of gastrointestinal smooth muscle

William C. Cole
The Smooth Muscle Research Group, Department of Physiology & Pharmacology, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada T2N 4N1

Email: wcole@ucalgary.ca

A major goal of research in the field of gastrointestinal (GI) physiology for more than the past half-century has been to understand the mechanisms responsible for control of smooth muscle motility by enteric motor neurotransmission. Morphologically defined synaptic structures, such as occur between neurons, and at skeletal muscle motor end-plates, are not evident between enteric nerves and smooth muscle cells within the GI tract. Rather, nerve varicosities are found in close apposition (within <20 nm) to non-muscle cell types, namely c-kit$^+$ interstitial cells of Cajal (ICC) and platelet-derived growth factor receptor α^+, fibroblast-like cells (referred to as FLC or PDGFRα^+ cells) (Daniel & Posey-Daniel, 1984; Komuro *et al.* 1999; Sanders *et al.* 2010), although close appositions to smooth muscle cells are also apparent. For this reason, other explanations have been sought to explain the transduction of motor impulses into changes in smooth muscle contractility. These include 'volume transmission' wherein transmitters are released into the interstitial space to affect multiple nearby smooth muscle cells (see Sarna, 2008), and transmission mediated via intermediary cell types, such as ICC (see Sanders *et al.* 2010 for a recent review) or FLC (Kurahashi *et al.* 2011), that are electrically coupled to smooth muscle cells via gap junctions. The results of several studies conducted during the past 20 years provide compelling evidence that ICC contribute to enteric neurotransmission, but this view is still questioned by some investigators (e.g. Sarna, 2008; Goyal & Chaudary, 2010).

In a recent issue of *The Journal of Physiology*, Zhu *et al.* 2011 provide significant new insights concerning the involvement of ICC in cholinerigic excitatory motor neurotransmission in the gut. Specifically, the post-junctional response to cholinergic stimulation within ICC of the murine small intestine is shown to involve the activation of niflumic acid-sensitive, Ca^{2+}-activated Cl^- channels owing to expression of the *mTmem16a* gene product, ANO1. These chloride channels are specifically expressed in ICC within the tunica muscularis of the GI tract (Gomez-Pinilla *et al.* 2009; Hwang *et al.* 2009; Zhu *et al.* 2009), and demonstration of their involvement in the response to muscarinic receptor activation represents a significant new brick in the wall of evidence supporting the view that ICC do indeed contribute to enteric excitatory motor neurotransmission.

ICC are known to express muscarinic M2 and M3 receptors (Chen *et al.* 2007) and are therefore potential targets of cholinergic excitatory nerves in the gut. Studies using transgenic models, such as W/W^V mice, indicate that cholinergic responses are suppressed in GI tissues in which ICC are absent or dramatically reduced in specific areas of the GI tract (e.g. gastric fundus, Ward *et al.* 2000). Such findings are consistent with the view that this specialized cell type plays an important intermediary role in cholinergic excitatory neurotransmission. However, it is well-accepted that GI smooth muscle cells also express muscarinic receptors and are depolarised by treatment with exogenous muscarinic agonists, such as carbachol, owing to the activation of non-selective cation channels (Benham *et al.* 1985; Inoue & Isenberg, 1990). Moreover, knock-out of TRPC4 and TRPC6 cation channels reduced cholinergic excitatory control in mouse ileal longitudinal muscle, consistent with a mechanism of neurotransmission that does not require an intermediary cell type (Tsvilovskyy *et al.* 2009). These observations are just two examples of the evidence supporting the opposing arguments for and against a role for ICC in GI motor transmission that are extensively considered in previous review articles (Sarna, 2008; Goyal & Chaudary, 2010; Sanders *et al.* 2010). If ICC do indeed play a role in cholinergic neurotransmission, they would be expected to exhibit a post-junctional response that would have a depolarising influence on adjacent smooth muscle cells. In this regard, a major piece of evidence that has been lacking until now has been the identity of the ionic conductance of ICC affected by cholinergic agonists and responsible for altering the electrical behaviour of surrounding smooth muscle via gap junction-mediated electrotonic coupling.

Zhu *et al.* (2011) show that the membrane conductance responsible for spontaneous transient inward currents (STICs) and their corresponding voltage responses, spontaneous transient depolarizations (STDs), in ICC derived from the deep muscular plexis, as well as the large, 'slow wave currents' of ICC from the myenteric plexis, is enhanced by muscarinic receptor activation in circular smooth muscle layer of the mouse jejunum. Carbachol treatment was found to: (i) enhance the frequency and amplitude of STICs and STDs, (ii) increase inward holding current at –80 mV and depolarize resting membrane potential, and (iii) decrease the rate of 'slow wave current' relaxation following repolarization to –80 mV, responses that were uniformly sensitive to pretreatment with muscarinic receptor antagonists. Unequivocal identification of ICC within the mixed population of cells isolated from the tissue by enzymatic dispersion in these experiments was facilitated by the use of transgenic mice in which green fluorescent protein is constitutively expressed within this cell type (i.e. the copGFP-ICC mouse; Zhu *et al.* 2009). Selective analysis of ICC in the deep muscular plexus was accomplished through the use of a novel cross of copGFP-ICC and W^V mice to specifically ablate GFP$^+$ ICC within the myenteric plexis. Identification of the ionic basis of the conductance affected by muscarinic stimulation was accomplished by determination of current reversal potential and the presence of appropriate changes in reversal potential following alterations in the equilibrium potential for chloride ions. The current was demonstrated to be Ca^{2+} sensitive, decreasing on reduction of Ca^{2+} concentration from 100 to 1 nmol l^{-1} at the intracellular face of excised, inside-out membrane patches, and to be blocked by the Ca^{2+}-activated Cl^- channel inhibitors, niflumic acid and

© 2011 The Author. Journal compilation © 2011 The Physiological Society
DOI: 10.1113/jphysiol.2011.218453
The Journal of Physiology

5-nitro-2-(3-phenylpropylamino)-benzoic acid. These findings on the native channels are supported by experiments demonstrating functional identity with recombinant channels. Specifically, Ca^{2+}-sensitive ANO1 channels were over-expressed in a human cell line (HEK293 cells) in combination with muscarinic M_3 receptors and shown to be activated by carbachol, but not when expressed in the absence of the receptors. Finally, the muscarinic receptor-dependent post-junctional response of intact mouse jejunum tissues to electrical stimulation, including depolarization and increased slow wave duration (as expected based on the membrane current data), but not the response to exogenous cholinergic agonist, was suppressed by niflumic acid block of Ca^{2+}-activated Cl^- channels. Niflumic acid was previously reported to affect TRPC4 channels (Walker *et al.* 2002), but this cannot account for the present results as it had no effect on the response to exogenous carbachol, i.e. the responses to electrical stimulation and exogenous agonist are mediated by different mechanisms. Taken together, these findings indicate for the first time that ANO1 Ca^{2+}-activated Cl^- channels of ICC are activated during cholinergic neurotransmission and contribute to the excitatory motor control of motility in the mouse jejunum.

The findings of Zhu *et al.* provide a unique insight concerning the identity of the molecular effector involved in the post-junctional response of the mouse small intestine to a major excitatory neurotransmitter. The use of the copGFP-ICC mice, as well as the ingenious cross of this model with the W^V mouse to permit a selective study of ICC from the deep muscular plexis, illustrate the power of combining classical electrophysiological analysis with genetic manipulation. It is now evident that ICC contribute to the post-junctional response to cholinergic excitatory nerves in mouse jejunum circular smooth muscle layer, but GI smooth muscle cells are clearly also sensitive to acetylcholine. Thus, it is likely that the total post-junctional response is a summation of different responses in at least these two cell types. Understanding how the varied electrical responses of ICC, PDGFRα^+ cells and smooth muscle cells, as well as the potential modulation of contractile filament Ca^{2+} sensitivity via biochemical signalling pathways involving Rho-associated kinase and protein kinase C, are integrated to control GI motility represents a significant challenge for the future. Success in this endeavour will clearly require a similarly detailed, thorough approach employing an extensive repetoire of research tools and animal models.

References

Benham CD, Bolton TB & Lang RJ (1985). *Nature* **316**, 345–347.

Chen H, Redelman D, Ro S, Ward SM, Ordoög T & Sanders KM (2007). *Am J Physiol Cell Physiol* **292**, C497–C507.

Daniel EE & Posey-Daniel V (1984). *Am J Physiol Gastrointest Liver Physiol* **246**, G305–G315.

Gomez-Pinilla PJ, Gibbons SJ, Bardsley MR, Lorincz A, Pozo MJ, Pasricha PJ *et al.* (2009). *Am J Physiol Gastrointest Liver Physiol* **296**, G1370–G1381.

Goyal RK & Chaudhury A (2010). *Am J Physiol Gastrointest Liver Physiol* **298**, G10–G13.

Hwang SJ, Blair PJ, Britton FC, O'Driscoll KE, Hennig G, Bayguinov YR *et al.* (2009). *J Physiol* **587**, 4887–4904.

Inoue R & Isenberg G (1990). *Am J Physiol Cell Physiol* **258**, C1173–C1178.

Komuro T, Seki K & Horiguchi K (1999). *Arch Histol Cytol* **62**, 295–316.

Kurahashi M, Zheng H, Dwyer L, Ward SM, Don Koh S & Sanders KM (2011). *J Physiol* **589**, 697–710.

Sanders KM, Hwang SJ & Ward SM (2010). *J Physiol* **588**, 4621–4639.

Sarna SK (2008). *Am J Physiol Gastrointest Liver Physiol* **294**, G372–G390.

Tsvilovskyy VV, Zholos AV, Aberle T, Philipp SE, Dietrich A, Zhu MX *et al.* (2009). *Gastroenterology* **137**, 1415–1424.

Walker RL, Koh SD, Sergeant GP, Sanders KM & Horowitz B (2002). *Am J Physiol Cell Physiol* **283**, C1637–C1645.

Ward SM, Beckett EA, Wang X, Baker F, Khoyi M & Sanders KM (2000). *J Neurosci* **20**, 1393–1403.

Zhu MH, Kim TW, Ro S, Yan W, Ward SM, Koh SD & Sanders KM (2009). *J Physiol* **587**, 4905–4918.

Zhu MH, Sung IK, Zheng H, Sung TS, Britton FC, O'Driscoll K, Koh SD & Sanders KM (2011). *J Physiol* **589**, 4565–4582.

© 2011 The Author. Journal compilation © 2011 The Physiological Society