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Ionic conductances regulating the excitability of colonic smooth muscles

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Abstract

The *tunica muscularis* of the gastrointestinal (GI) tract contains two layers of smooth muscle cells (SMC) oriented perpendicular to each other. SMC express a variety of voltage-dependent and voltage-independent ionic conductance(s) that develop membrane potential and control excitability. Resting membrane potentials (RMP) vary through the GI tract but generally are within the range of -80 to -40 mV. RMP sets the 'gain' of smooth muscle and regulates openings of voltage-dependent Ca^{2+} channels. A variety of K^{+} channels contribute to setting RMP of SMC. In most regions RMP is considerably less negative than the K^{+} equilibrium potential, due to a finely tuned balance between background K^{+} channels and non-selective cation channels (NSCC). Variations in expression patterns and openings of K^{+} channels and NSCC account for differences of the RMP in different regions of the GI tract. Smooth muscle excitability is also regulated by interstitial cells (interstitial cells of Cajal (ICC) and $\text{PDGFR}\alpha^{+}$ cells) that express additional conductances and are electrically coupled to SMC. Thus, 'myogenic' activity results from the integrated behavior of the SMC/ICC/ $\text{PDGFR}\alpha^{+}$ cell (SIP) syncytium. Inputs from excitatory and inhibitory motor neurons are required to produce the complex motor patterns of the gut. Motor neurons innervate three cell-types in the SIP, and receptors, second messenger pathways and ion channels in these cells mediate post-junctional responses. Studies of isolated SIP cells have begun to unravel the mechanisms responsible for neural responses. This review discusses ion channels that set and regulate RMP of SIP cells and how neurotransmitters regulate membrane potential.

Keywords

ion channels; smooth muscle; enteric nervous system; interstitial cells of Cajal; $\text{PDGFR}\alpha^{+}$ cells; colonic motility; excitation-contraction coupling

I. INTRODUCTION

Colonic smooth muscles generate contractile behaviors that facilitate recovery of water and electrolytes from the proximal colon, and propulsive contractions to evacuate feces. Colonic migrating motor complexes (CMMC) are large amplitude, propulsive contractions initiated by action potential complexes that propagate along the length of the colon (0.3 cycles per min in mouse).^{1,2} Slow waves generated by interstitial cells of Cajal initiate action potential complexes, and neural inputs regulate the frequency and force of contractile responses. Action potentials in colonic smooth muscle cells result from the activation of voltage-

dependent Ca^{2+} channels. Depolarization enhances the open probability of Ca^{2+} channels (α pore subunits encoded by *Cacna1c* and *Cacna1h*) (see Table 2).^{3,4} Small depolarizations (<10 mV) initiate regenerative activation of Ca^{2+} channels producing action potentials from resting potentials of colonic smooth muscle cells. The number and frequency of action potentials per complex is determined dynamically by the relative open probabilities of outward current conductances provided by a variety of K^+ channels.⁵ These include voltage- and Ca^{2+} -dependent K^+ channels (encoded in smooth muscle cells mainly by *Kcna2*, *Kcna5*, *Kcnb2*, *Kcnh2*, *Kcnd3*, and *Kcnma1*) (see Table 1).

Resting membrane potentials (RMP) of colonic muscles (~ -50 mV) are considerably less negative than the K^+ equilibrium potential ($E_{\text{K}} = \sim -80$ mV). Thus, the resting K^+ conductance is balanced by conductances that generate inward currents. It is unlikely that voltage-dependent Ca^{2+} channels provide the inward current necessary for producing membrane potentials 30 mV less negative than E_{K} because neither the L-type Ca^{2+} channel blocker, nifedipine, nor T-type channel blocking ion, Ni^{2+} , affect resting potentials. It appears that background inward currents in colonic muscles are due to non-selective cation channels and possibly Ca^{2+} -activated Cl^- channels. Regulation of these conductances is an important factor in setting the excitability of colonic muscles.

A common theme in the GI tract is that the open probability of voltage-dependent Ca^{2+} channels in GI smooth muscle cells is not regulated directly by the many bioagonists that influence smooth muscle contractions. Instead, Ca^{2+} channel open probability is controlled by transmembrane potential, and this is regulated by a plethora of ionic conductances that are, in turn, regulated by neurotransmitters and other regulatory hormonal and paracrine agonists, stretch, pH, intracellular Ca^{2+} ($[\text{Ca}^{2+}]_{\text{i}}$), G proteins, and several intracellular protein kinases. Smooth muscle cells also display spontaneous transient currents that depend upon factors such as Ca^{2+} release from intracellular stores. Summation of currents carried by these conductances within thousands of cells making up the smooth muscle syncytium yields ongoing net inward or outward currents and contributes to the regulation of excitability. Both spontaneous transient outward currents (STOCs) and spontaneous transient inward currents (STICs) have been reported in colonic smooth muscle cells. This review will describe many of the regulatory conductances in colonic muscles and their effects on membrane excitability, open probability of voltage-dependent Ca^{2+} channels and excitation-contraction coupling (see Table 1–3).

The opening of Ca^{2+} channels in smooth muscle cells results in Ca^{2+} influx (see Table 2). A large increase in the open probability of these channels produces action potentials and raises $[\text{Ca}^{2+}]_{\text{i}}$ to levels sufficient, via binding to calmodulin, to activate myosin light chain kinase (MLCK) and phosphorylation of myosin (i.e. excitation-contraction coupling). Thus, the multiple action potentials in a propagating CMMC initiate strong propulsive contractions. Contractile force is further regulated by balancing myosin phosphorylation via myosin phosphatase (MLCP). This important process, known as Ca^{2+} sensitization/desensitization, is reviewed in detail elsewhere.⁶

The excitability of colonic smooth muscles is not determined solely by conductances intrinsic to smooth muscle cells, because these cells are electrically coupled via gap

junctions to interstitial cells (i.e. interstitial cells of Cajal (ICC) and PDGFR α ⁺ cells),⁷ forming a smooth muscle cell/ICC/PDGFR α ⁺ cell (SIP) syncytium (Figure 1). Interstitial cells also express conductances that can be regulated by intrinsic factors, neurotransmitters and other regulatory agonists. A particularly important contribution of interstitial cells is spontaneous activation of ionic conductances that influence the excitability of the SIP syncytium. ICC, for example, generate electrical slow waves that contribute to the generation and timing of CMMC in colonic muscles. ICC also generate STICs that lead to spontaneous transient depolarizations (STDs)⁸ an ongoing instability in colonic membrane potential that enhances excitability.⁹ The occurrence of STDs is one reason why the threshold for Ca²⁺ action potentials is so close to the net resting potentials of colonic muscles. PDGFR α ⁺ cells generate STOCs that produce net outward currents in colonic smooth muscles and tend to counter balance depolarizing conductance(s).¹⁰

Superimposed upon the intrinsic behaviors of cells making up the SIP syncytium are the regulatory functions of neurotransmitters released from enteric motor neurons, hormones, paracrine substances and inflammatory mediators, many of which affect the open probabilities of ionic conductances of SIP cells (Figure 2). Neurotransmitters and bioactive agonists that activate net inward currents in any cell of the SIP syncytium will tend to depolarize the syncytium, increase net Ca²⁺ channel open probability in smooth muscle cells and facilitate excitation-contraction coupling. Activation of net outward current in any SIP cell will tend to hyperpolarize the SIP syncytium, decrease Ca²⁺ channel open probability in smooth muscle cells, inhibit action potentials and reduce excitation-contraction coupling.

As in many regions of the GI tract, colonic myogenic activity is tonically suppressed by intrinsic (enteric) inhibitory neural activity that releases nitric oxide (NO)¹¹ and a purine, most likely β -nicotinimide adenine dinucleotide (β -NAD).¹² The neurotoxin, tetrodotoxin (TTX) causes depolarization of membrane potential of colonic muscles, enhanced action potentials, and contraction.¹³ Specific deletion of nitrergic input with a nitric oxide synthase (NOS) inhibitor or purinergic input with apamin, to block a portion of the post-junctional response, also enhances electrical and mechanical activity. Tonic inhibition of myogenic activity may be an extremely important feature of colonic behavior and serve to suppress uncoordinated contractile activity or development of tone between propulsive contractions.

Stimulation of intrinsic nerves results in complex membrane potential changes because both excitatory and inhibitory motor neurons are activated. When excitatory nerves are blocked (at <10 Hz of stimulation this can be accomplished by muscarinic antagonists), only inhibitory junction potentials (IJPs) remain. In the colon, IJPs are composed of two components, a fast hyperpolarization (fIJP) response due to the purine neurotransmitter and a slower, smaller amplitude hyperpolarization (sIJP) due to NO.^{14,15} Recent studies have shown that the purinergic response is mediated via P2Y1 receptors.^{15,16} When intrinsic nerves are stimulated (<10 Hz) in the presence of a P2Y1 antagonist (e.g. MRS2500) and NOS inhibitors, a cholinergic depolarization occurs that is sustained throughout the duration of stimulation. The ionic conductances linked to regulation of RMPs in colonic muscles are discussed in this review. Additional conductances involved in generating and regulating colonic excitability are summarized in the tables (Table 1–3).

An interesting observation that has emerged from studies of isolated SIP cells is that neurotransmitters elicit responses by different mechanisms in these cells. As an example, muscarinic agonists, by binding to M_2 and M_3 receptors, activate, among other conductances, a non-selective cation conductance in smooth muscle cells.^{17,18} Cholinergic neurotransmission activates Ca^{2+} -activated Cl^- channels, expressed by ICC, by binding to M_3 receptors.⁸ Activation of both of these conductances yields inward currents and a tendency for depolarization of the SIP syncytium. It should be recognized, therefore, that studies demonstrating the receptors, intracellular signaling pathways, and conductances activated by exogenous neurotransmitters may not be reflective of the receptors, signaling pathways and conductances activated during enteric motor neurotransmission. At present, there is controversy about the role of ICC in motor neurotransmission.^{7,19} Characterizing the responses of SIP cells to neurotransmitters and then determining which receptors and effectors are utilized during responses to motor neurotransmission may provide the means for solving this controversy. We believe that inducible, cell-specific knockouts will be the best way to establish which cells are functionally innervated by motor neurons in the GI tract. Identifying the specific receptors and effectors in the SIP syncytium utilized by neurotransmitters may provide specific strategies for therapeutic modulation of motor neurotransmission and control of smooth muscle excitability.

II. K^+ conductances regulating resting membrane potential of the SIP syncytium

a. Small conductance Ca^{2+} -activated K^+ channels (SK channels)

Apamin, a peptide neurotoxin from bee venom, blocks small conductance Ca^{2+} -activated K^+ (SK) channels expressed in the GI smooth muscles. Apamin causes depolarization (~ 10 mV) and augmentation of action potentials and contractions in colonic muscles (see Table 3). Thus, SK channel openings occur under basal conditions in colonic muscles and contribute to the regulation of resting membrane potential. There is evidence that openings of SK channels are linked to tonic activity of purinergic enteric inhibitory nerves because spontaneous inhibitory junction potentials (IJP) are noted in intracellular recordings from colonic muscles. Apamin and TTX abolish the spontaneous IJP and cause depolarization.²⁰

Evaluation of SK expression in colon muscles showed SK2 (*Kcnn2*) to be the dominant isoform expressed by smooth muscle cells.^{21,22} Smooth muscle cells displayed modest apamin-sensitive currents when exposed to purines.^{23,24} However, another cellular component of the SIP syncytium, PDGFR α^+ cells, express SK3 robustly (*Kcnn3*).^{25–27} After immunological identification of PDGFR α^+ cells became possible,²⁶ an animal with eGFP expressed in PDGFR α^+ cells was utilized to isolate these cells for electrophysiological studies of their responses to purines.²⁷ Large amplitude, apamin-sensitive outward currents were elicited by purines and P2Y1 receptor agonists in PDGFR α^+ cells. When the effects of purines on smooth muscle cells and PDGFR α^+ cells were compared, using physiological gradients and holding potentials that approximated resting membrane potentials in situ, outward currents were generated in PDGFR α^+ cells and small inward currents were generated in smooth muscle cells (see Table 3). Thus, purinergic hyperpolarization

responses (IJP) in whole muscles are likely to be generated by PDGFR α ⁺ cells rather than by smooth muscle cells.

b. ATP-sensitive K⁺ channels (K_{ATP} channels)

The K_{ATP} openers (leimakalim, cromakalim, pinacidil, diazoxide etc) and a K_{ATP} blocking drug (glibenclamide) have been used to test the function of K_{ATP} channels in colonic smooth muscles. Lemakalim or cromakalim caused hyperpolarization and inhibition of spontaneous contractile activity. These effects were blocked by glibenclamide in intact muscles.²⁸ The effects of glibenclamide on the RMP vary depending on species. For instance, glibenclamide depolarized murine colonic muscles²⁸ but not canine colonic muscle.²⁹ Furthermore, H₂S, a neurotransmitter candidate in the GI tract, decreased spontaneous contractility in human colonic muscles and these effects were inhibited by glibenclamide suggesting that K_{ATP} channels could be a mediator of H₂S-dependent hyperpolarization. (see Table 3).

In patch clamp experiments, lemakalim and pinacidil activate K_{ATP} currents that are blocked by glibenclamide in murine colonic myocytes. Intracellular application of ADP (1 mM) or ATP (0.1 mM) reactivated channel openings after run-down under the excised (inside-out) patch configuration.²⁸ K_{ATP} channels are regulated by cAMP-dependent protein kinase (PKA) in visceral smooth muscles. For example, forskolin, isopreterenol, vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase activating-peptide (PACAP) activate K_{ATP} channels and induce hyperpolarization. Thus, K_{ATP} could be one of the ionic conductances responsible for the effects of inhibitory peptide neurotransmitters in colonic muscles. These peptides bind to G-protein coupled receptors and activate of ion channels via G α_s , activation of adenylate cyclase, production of cAMP, and protein kinase A. In contrast, protein kinase C inhibits K_{ATP} channels. Acetylcholine (ACh) suppresses K_{ATP} channels via activation of PKC in smooth muscle cells, and PKC ϵ appears to be the major isozyme regulating K_{ATP} channels in murine colonic myocytes.³⁰ In a study of the role of K_{ATP} channels in inflammatory bowel disease, dextran sulphate sodium (DSS)-treated mice had augmented lemakalim-activated K_{ATP} currents in comparison to control mice,³¹ suggesting that inflammation may influence the expression of K_{ATP} channels. Thus, K_{ATP} channels may be involved in regulation of RMP in physiological and pathological conditions affecting colonic excitability.

K_{ATP} channels are formed from a complex of pore-forming subunit *Kcnj8* (Kir 6.1) and *Kcnj11* (Kir 6.2) and sulfonylurea receptors (SUR1 and SUR2). RT-PCR demonstrated expression of Kir 6.1 and Kir 6.2 and SUR2B transcripts in colonic smooth muscle cells.^{28,32} Kir 6.1 subunits were found to be localized at the plasma membrane, whereas Kir 6.2 protein was detected in the cytosol by immunohistochemistry. Co-expression of SUR2B and Kir6.1 or 6.2 subunits resulted in functional K_{ATP} channels in colonic smooth muscle cells.³³ Interestingly, quantitative analysis of PCR products showed that Kir 6.1 expression was increased, but SUR2B was decreased, in a murine model of colitis.³² These transcriptional changes may be related to the decreased colonic motility observed in models of inflammation.

Expression of K_{ATP} channels in colonic ICC or $PDGFR\alpha^+$ cells has not been reported to date. A few studies have reported K_{ATP} channels in small intestinal ICC, but expression of K_{ATP} expression in colonic ICC has not been documented. ACh inhibits K_{ATP} channels in smooth muscle cells, but there have been no reports of that nerve-evoked excitatory junction potentials elicited by ACh are absent in colonic muscles in the presence of glibenclamide. Further studies are needed to determine whether there is functional expression of K_{ATP} channels in colonic ICC and/or $PDGFR\alpha^+$ cells and whether these openings of these channels is modulated by neurotransmitters. Activation or suppression of K_{ATP} channels in response to neurotransmission may be an interesting method of determining which cells mediate responses to transmitters released from enteric motor neurons.

c. Inwardly rectifying K^+ channels (Kir channels)

Kir conductances contribute to membrane potentials because outward currents are generated via these channels near the resting membrane potential of colonic muscles. Low concentrations of Ba^{2+} (μM range) have been used to investigate the function of Kir channels. In canine colonic muscles, Ba^{2+} (1–100 μM) depolarized cells along the submucosal surface of the circular muscle layer. When the submucosal and myenteric pacemaker regions were surgically removed, higher concentrations of Ba^{2+} were required to depolarize circular muscle. Patch-clamp recordings revealed higher current density of a Ba^{2+} -sensitive Kir conductance in ICC than that in smooth muscle cells. Quantitative PCR showed greater expression of *Kcnj2* (Kir2.1) mRNA along the submucosal surface of the circular muscle layer. These data suggest that colonic ICC express Ba^{2+} -sensitive Kir2.1 channels that might contribute to the negative resting potentials of cells near the submucosal surface of the circular muscle (see Table 3).³⁴

Another member of the Kir channel family has also been reported in colonic smooth muscle cells. G-protein-gated inwardly rectifying K^+ channels (GIRK channels) are highly expressed in neurons and heart and mediate inhibitory effects of many neurotransmitters.³⁵ GIRK channels hyperpolarize these tissues in response to binding of agonists to G protein-coupled receptors. A prominent biophysical property of GIRK channel is inward rectification due to blockade by internal Mg^{2+} ions and positively charged polyamines. Due to this block by extrinsic factors GIRK channels demonstrate voltage-dependent properties without an intrinsic voltage sensor. In cells under physiological conditions GIRK produces small outward currents at membrane potentials slightly less negative than E_K .³⁶

Kcnj3 (Kir3.1) and *Kcnj6* (Kir3.2) transcripts were found in canine colonic smooth muscle cells by PCR, and expression of both proteins was confirmed by immunohistochemistry.³⁷ The functional role of GIRK channels has not been demonstrated either in tissue experiments or by experiments on isolated smooth muscle cells. In the colon, ACh activates non-selective cation channel (NSCC, see below), but GIRK channels may also be activated. NSCC tends to depolarize the muscles, and GIRK channels may provide an opposite current to help maintain negative resting potentials between electrical slow waves. ACh responses wane during sustained stimulation, but this can be attributed to desensitization of muscarinic receptors and/or breakdown of ACh by acetylcholine esterase.

d. K2P channel (TREK and TASK channels)

Stretch-dependent two pore domain (K2P) K⁺ channels are expressed by colonic smooth muscle cells.^{38,39} Currents via these channels are part of the background whole-cell conductance, and thus K2P channels contribute to RMPs of colonic muscles. Colonic smooth muscle cells experience stretch during luminal filling and contractions. Cell elongation or stretch of many smooth muscle cells activates inward currents due to presence of mechano-sensitive non-selective cation channels⁴⁰ and stretch activation of L-type Ca²⁺ channels.⁴¹ Stretch of colonic muscles, however, does not cause depolarization. This is an important property of the proximal colon and helps this region fulfill a reservoir function to facilitate recovery of water and electrolytes. The ability increase luminal diameter during filling without stimulation of contraction is due, in part, to the expression of stretch-dependent K⁺ (SDK) channels. Activation of SDK channels counterbalances inward currents through NSCC and stabilizes membrane potential.

K2P family channels encoded by *Kcnk2* (TREK-1), *Kcnk10* (TREK-2), and *Kcnk4* (TRAAK) are stretch-dependent. Murine colonic smooth muscle cells express TREK-1 channels.^{38,39} Homologous expression of TREK-1 in COS-7 cells resulted in channels with a unitary conductance similar to the K⁺ channels expressed in smooth muscle cells. Channels with this unitary conductance were activated in smooth muscle cells in response to cell elongation.³⁹ These data suggest that TREK-1 channel mediates the SDK conductance in colonic smooth muscles. It should also be noted that the SDK conductance in colonic myocytes is also activated by nitric oxide via a cGMP and protein kinase G-dependent mechanism.^{38,39} Thus, SDK channels may also be involved in mediation of nitroergic hyperpolarization responses in colonic muscles (see Table 3).

Studies to identify the role of SDK channels were frustrated by the lack of specific blockers for these channels. We found that sulfur-containing amino acids, e.g. L-methionine, provided a relatively specific blocker of SDK channels in colonic smooth muscle cells. These amino acids also blocked TREK-1 channels expressed in COS-7 cells. L-methionine and a derivative, methioninol, have been tested on colon, bladder and uterine smooth muscles to determine the role of TREK-1 expression in smooth muscle organs specializing in volume storage.⁴²⁻⁴⁴ L-methionine induced depolarization and increased spontaneous contraction in the colon, providing evidence that basal activation of TREK-1 (SDK) channels stabilizes the excitability of colonic muscles. It should be noted that since the colon is under tonic inhibitory drive provided by inputs from nitroergic enteric inhibitory neurons,¹³ NO may be the stimulus for basal activation of TREK-1 channels. Inhibition of NO synthesis in colonic muscles causes depolarization and increased spontaneous contractions, similar to the effects of L-methionine.

In addition to L-methionine and its derivatives, several Ca²⁺ store-active compounds, including caffeine, ryanodine, and cyclopiazonic acid, also inhibit TREK-1 channels.⁴⁵ The blocking effect of caffeine on TREK-1 channels is likely due to its actions as an inhibitor of phosphodiesterases, preservation of cAMP, and activation of protein kinase A. TREK-1 channels are inhibited by phosphorylation of serine 333, which is a target for PKA in TREK-1. Observing the effects of Ca²⁺ store-active compounds on nitroergic inhibitory

junction potentials (IJs) has led some authors to conclude that IJs are due to Ca^{2+} dependent conductances.⁴⁶ However, the effects of these compounds could be due to their inhibitory effects on TREK-1 channels, which are activated by NO, as described above. The role of TREK-1 channels in GI muscles is still controversial, however, and there may be tissue and species specific differences in expression of these channels and their role in mediating nitroergic responses. For example, L-methionine caused depolarization of mouse and opossum lower esophageal sphincter (LES) but nitroergic IJs were not blocked in these muscles.⁴⁷ Smooth muscle specific knockouts of TREK-1 are needed to solve this controversy. However, there are inherent problems in obtaining offspring from mice with constitutive knockouts in TREK-1, because these channels also have an important role in stabilizing the excitability of the uterus and maintaining pregnancy to term.⁴³

Another K2P channel, TASK channel, also contributes to background K^+ conductance and setting of membrane potential in GI muscles. TASK-2 (*Kcnk5* or K2P5.1) channel cloned from murine intestinal muscles, is a pH-sensitive, time-dependent, non-inactivating K^+ conductance with slow activation kinetics. These channels are inhibited by local anesthetics. A conductance with kinetics and pH-sensitivity to TASK channel is apparent in colonic myocytes. Lidocaine, bupivacaine and acidic pH depolarized colonic circular muscle cells.⁴⁸

III. Inward current conductances

a. Non-Selective Cation Channels (NSCC)

The majority of studies of NSCC in GI smooth muscle cells have focused on the conductance activated by muscarinic stimulation. Muscarinic receptors (M_2 and M_3) mediate enteric excitatory motor neurotransmission.⁴⁹ Muscarinic receptors in smooth muscle cells are coupled via G-proteins⁵⁰ to activation of a non-selective cation current (called mI_{cat}). Activation of mI_{cat} results in inward current and depolarization of smooth muscle cells. Opening of mI_{cat} channels may allow entry of some Ca^{2+} , but in physiological gradients, it appears that these channels favor Na^+ as the charge carrier. Depolarization, however, results in activation of voltage-dependent Ca^{2+} channels, Ca^{2+} entry, and contraction. Neurokinin A and substance P (via NK1 or NK2 receptors) also depolarize colonic myocytes by activation of a NSCC.⁵¹

An important function of NSCC in colonic smooth muscles is the setting of membrane potential and excitability. Spontaneous openings of NSCC occur on an ongoing basis, and openings of clusters of NSCC generate STICs in smooth muscle cells.⁵² Resting membrane potentials of smooth muscles in all parts of the GI tract are less negative than the equilibrium potential for K^+ (E_{K}) ions. In addition to the high permeability for K^+ , substantial permeability must exist for ions carrying inward current. The inward current conductances appear to be due to NSCC that primarily use Na^+ as the charge carrier. Colonic muscles are polarized at rest at a level that is quite close to the activation range for voltage-dependent Ca^{2+} channels. Thus, a small increase in the open probability of NSCC can affect spontaneous electrical activity (e.g. generation of action potentials), responses to neurotransmitters (e.g. whether a small depolarization event caused by an excitatory junction potential initiates an action potential) and contractile activity. NSCC pre-condition and set the gain for responses of colonic muscles.

We have described the basal activity of NSCC in human and monkey colonic myocytes.⁵² Replacement of extracellular Na^+ with equimolar tetraethylammonium or Ca^{2+} with Mn^{2+} inhibited basal NSCC openings under voltage-clamp. Under current clamp, replacement of extracellular Na^+ with N-methyl-D-glucamine (NMDG) resulted in hyperpolarization. The pharmacology of the native NSCC is complicated and there are no specific blockers for these channels. Trivalent cations are typically used to block NSCC. Application of trivalent cations (La^{3+} and Gd^{3+}) to colonic muscles and myocytes blocked NSCC and caused hyperpolarization. These findings strongly support the hypothesis that ongoing openings of NSCC contribute to setting of membrane potential and regulation of excitability in colonic muscles.

NSCC in colonic smooth muscle cells appear to be due to expression of various transient receptor potential (TRP) channels. Seven main subfamilies make up TRP superfamily on the basis of their structural homology: TRPC (Canonical), TRPV (Vanilloid), TRPM (Melastatin), TRPP (Polycystin), TRPML (Mucophilin), TRPA (Ankyrin) and TRPN (no mechanoreceptor potential C, NOMPC). Functional TRP channels are composed of either homo- or heteromultimers of four TRP subunits, and the short hydrophobic stretch between transmembrane domain 5 and 6 is thought to form the cation-permeable pore. Qualitative PCR revealed expression of TRPC1, C3, C4, C7, M2, M4, M6, M7, V1, and V2 in human and monkey colonic myocytes. TRPM4 and specific heteromultimeric combinations of TRPC channels may underlie the basally active NSCC in colonic smooth muscle cells based on single channel conductances.¹⁸ Transgenic mice with specific lesions in TRP channels will be needed to sort out the specific role for each ion channel species in regulation of colonic excitability. For example, a recent gene deactivation study demonstrated that mI_{cat} results from co-expression of TRPC4 and TRPC6 in GI smooth muscle cells.¹⁸

b. Ca^{2+} -activated Cl^- channels (CaCC)

The expression of CaCC in GI smooth muscles was a major debate for several years. Several investigators tested the effects of unspecific chloride channel blockers (niflumic acid, DIDS, 9-AC etc) on membrane potential or contractility and interpreted results to be evidence that CaCC might regulate resting potentials and/or responses to neurotransmitters. For instance, niflumic acid caused hyperpolarization and inhibited NK-1 agonist-induced contraction in rabbit colon.⁵³ Now the non-specificity of many Cl^- channel blocking drugs is widely recognized, and it is possible that effects, particularly of niflumic acid, could be mediated by block of NSCC or other conductances. Only a few reports of CaCC have emerged after many years of patch clamp studies of GI smooth muscle cells,^{54,55} which suggests that channels of this type are limited to certain muscles or species.

More recent studies of CaCC in the gut have focused on expression of transcripts of *Tmem16a* (ANO1). ANO1 has been identified as a CaCC, and there is abundant expression of these channels in the *tunica muscularis*. We reported that *Tmem16a* transcripts were highly expressed in ICC in a genome-wide expression study of ICC-MY and ICC-DMP from the small intestine (see Table 2).⁵⁶ Immunohistochemistry confirmed that ANO1 channel proteins are expressed in ICC specifically throughout the GI tract. These studies also demonstrated that expression is low or absent in smooth muscle cells.^{57,58}

ICC generate electrical pacemaker activity (slow waves) in GI muscles.⁵⁹ CaCC blocking drugs, niflumic acid and 4,4-diisothiocyano-2,2-stillbene-disulfonic acid (DIDS) blocked slow waves in murine, primate, human small intestine and stomach,⁵⁸ however, as stated above, most chloride channel blockers are not highly specific. Most studies of isolated ICC had relied upon a cultured cell model, and the phenotype of these cells changes dramatically within a few days. We developed a transgenic mouse with a bright green reporter (copGFP) expressed in ICC,⁶⁰ so freshly dispersed ICC could be identified and studied selectively. ICC from these animals generate slow wave currents and slow wave-like depolarizations.⁶¹ Channels with a conductance similar to expressed ANO1 (8pS) were observed in ICC. ICC generated spontaneous large amplitude transient depolarizations (slow waves) in current-clamp that were blocked by niflumic acid. The slow waves in single cells corresponded to large inward currents recorded under voltage-clamp.⁶² Transgenic animals in which *Tmem16a* was deactivated lacked slow waves in GI muscles. Thus, CaCC appear to be responsible for spontaneous pacemaker activity in the GI tract.

Another feature common to many GI muscles is the presence of an ongoing discharge of spontaneous transient depolarizations (STDs).^{9,62} David Hirst referred to these events as 'unitary potentials', and described the stochastic nature of the discharge of these events in GI muscles. We do not favor the term unitary potentials because this term may imply that the voltage fluctuations result from 'unitary currents' (a term referring to the current through a single ion channel). STDs are of sufficient amplitude that they must result from the coincident openings of many channels. STDs provided by openings of CaCC channels in ICC exert a net inward current on the SIP syncytium. The events underlying STDs (spontaneous transient inward currents or STICs) are also likely to be the underlying pacemaker mechanism for slow waves.

In colonic smooth muscles, immunohistochemistry confirms that all populations of ICC express ANO1. Colonic muscles from *W/W^v* mice display prominent reductions in post-junctional responses to excitatory and inhibitory neurotransmission.⁷ Slow wave activity and normal spontaneous action potential complexes are also reduced in these animals.⁷ Membrane potential recordings also demonstrate loss of the ongoing discharge of STDs.⁷ Thus, loss of ICC that carry expression of ANO1, greatly reduces electrical rhythmicity, blocks slow wave activity and reduces post-junctional neural responses. These data demonstrate that CaCC are an important conductance in regulation of colonic electrical activity.

III. CONCLUSIONS

An important concept that has emerged regarding the 'myogenic' activity of the gut is that ionic conductances in at least 3 cell-types contribute to the regulation of GI smooth muscle electrical excitability. Patch clamp experiments on smooth muscle cells, ICC and PDGFR α ⁺ cells have begun to clarify the conductances present and delineate the conductances that regulate excitability and produce the electrical activity manifest at the tissue level (Figure 2). Smooth muscle cells, ICC and PDGFR α ⁺ cells are interconnected, forming an electrical syncytium, referred to in this review as the SIP syncytium. ICC generate electrical slow waves via activation of CaCC channels. Slow wave depolarizations are conducted to smooth

muscle cells that respond to pacemaker activity with activation of voltage-dependent Ca^{2+} currents and K^{+} currents. These conductances yield action potentials, Ca^{2+} entry, and initiation of contraction.

Another concept described recently, is the regulation of membrane potential provided by the stochastic occurrence of transient currents in cells of the SIP syncytium. Smooth muscle cells express NSCC that undergo periodic openings of clusters of channels (STICs) and low amplitude STDs. Smooth muscle cells also express Ca^{2+} -activated K^{+} channels (BK and SK channels) that can also display bursts of channel openings and spontaneous transient outward currents (STOCs). STOCs due to BK channels are uncommon in colonic smooth muscle cells at the level of resting $[\text{Ca}^{2+}]_i$ and at the resting membrane potentials of these cells *in situ*. STOCs, due to SK channels, also occur, but these are of very low amplitude at the resting potentials of smooth muscle cells. STICs and STOCs, generated by thousands of smooth muscle cells in the syncytium, provide a net influence on membrane potential. $\text{PDGFR}\alpha^{+}$ cells have a high density of SK3 channels and tonic input from inhibitory neurons. Thus, these cells also contribute to membrane potential through generation of spontaneous inhibitory junction potentials (IJP) and STOCs that are independent of neural inputs, as recorded in isolated $\text{PDGFR}\alpha^{+}$ cells. ICC display large amplitude STICs resulting from periodic activation of clusters of CaCC. These events yield STDs that are likely to be the fundamental pacemaker mechanism underlying slow waves. STDs cause ongoing noisy resting potentials in intact muscles, and muscles from animals lacking ICC display significant reduction in this noise and are frequently hyperpolarized relative to wildtype muscles.⁷⁷ Activation of clusters of ion channels in cells of the SIP syncytium and generation of STICs and STOCs results from transient, compartmentalized Ca^{2+} release events from intracellular Ca^{2+} stores.

Slow waves, STICs and STOCs, and voltage-dependent conductances in cells of the SIP syncytium (and therefore resting membrane potentials) are modulated by inputs from enteric excitatory and inhibitory nerves, which appear to innervate all 3 types of cells. ICC appear to have a role in mediating cholinergic and nitrergic responses; $\text{PDGFR}\alpha^{+}$ cells appear to mediate purinergic inhibitory responses. Smooth muscle cells also express receptors and ion channels that bind to and are responsive to neurotransmitters. Thus, transduction of neural inputs by smooth muscle cells may also contribute to post-junctional responses.

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Figure 1.

Relationship between smooth muscle cells (SMC), intramuscular interstitial cell of Cajal (ICC-IM) and PDGFR α ⁺ cells (fibroblast-like cell; FLC). A: SMC, ICC-IM and PDGFR α ⁺ cells are closely associated with nerve bundles (NB), as in this section from rat stomach (A; scale bar is 0.5 μ m; reproduced with kind permission from Springer Science+Business Media: Mitsui & Komuro, 2002, *Cell & Tissue Res*; **309**: 219–227). Both ICC-IM and PDGFR α ⁺ cells form close contacts with nerve varicosities(*). Inset (scale bar 0.2 μ m) denotes by the asterisk region in panel A. B–D: Double labeling of PDGFR α ⁺ cells (green)

and ICC-IM (red) show these cells are discrete phenotypes but occupy similar anatomical spaces in the human colon. *E–G*: multipolar PDGFR α^+ -MY cells (green; arrows) are closely associated with ICC-MY (red) in the plane of the myenteric plexus of human colon. Scale bar denotes 40 μ m. (Kurahashi M *et al*, Cell Mol Med. 2012, e-pub ahead)

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A. Triumvirate of myogenic excitability

B. Human colonic circular smooth muscle

C. Monkey colonic circular smooth muscle

D. Murine colonic circular smooth muscle

Figure 2.

'Myogenic' activity in GI muscles is the result of electrical activities of at least 3 electrically coupled cell types: smooth muscle cells, interstitial cells of Cajal, PDGFR α ⁺ cells (Panel A). Cartoon depicts SIP syncytium: (SMC; green, ICC; blue, PDGFR α ⁺; red) and shows major conductances setting resting membrane potentials, generating slow waves and action potentials, and mediating neural responses in colonic muscles. B–D: Human (panel B), monkey (panel C) and mouse (panel D) show similar electrical patterns in colonic muscles, with spontaneous generation of slow waves and spike complexes. EFS in panel D denotes

electrical field stimulation (0.3ms, 5Hz, 150V for 5sec train duration). EFS evoked an excitatory junction potential (cholinergic responses) followed by fast (purinergic response) and slow (nitrgergic response) inhibitory junction potentials.

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Table 1

Voltage-dependent K⁺ channels in colonic smooth muscle

	Large Conductance Ca ²⁺ -activated K ⁺ Channel	Delayed Rectifying K ⁺ Channel	A-type K ⁺ Channel	Human ether-a-go-go-related Channel	Slowly Delayed Rectifying K ⁺ Channel
Gene candidate	<i>KCNMA1</i>	<i>KCNA2</i> , <i>KCNA5</i> , <i>KCNB2</i>	<i>KCND1</i> , <i>KCND2</i> , <i>KCND3</i>	<i>KCNH2</i>	<i>KCNQ3</i> , <i>KCNQ4</i>
IUPHAR name	KCa1.1	Multiple candidates; Heteromultimer Kv1.2, Kv1.5, Kv2.2	Kv4.1, Kv4.2, Kv4.3	Kv11.1	Kv7.3, Kv7.4
Other names	BK, K _{Ca} , slo, slo1	K _{DR}	K _A , I _{to}	HERG, egr1	none
Expressed cell type	Smooth muscle cell	Smooth muscle cell	Smooth muscle cell	Smooth muscle cell	Smooth muscle cell
Property	Voltage-dependent and Ca ²⁺ -dependent	V _a * = -45mV, Slow inactivation	Low-voltage activated (-55mV), Fast inactivation	During repolarization resurgent current due to recovery from C-type inactivation	
Unitary conductance	200–300pS	10–20pS	19pS	2pS based on expressed cell, 5–10pS	?
Modulator/Agonist	Tamoxifen, Estrogen	VIP, Isoproterenol	CaMKinase II slow inactivation	RPR26023	
Antagonist/Blocker	Charybdotoxin Iberitoxin, TEA	TEA, 4AP (> 3mM) Dendrotoxin, Margatoxin, Nuxitoxin	4-AP (<3mM), Flecainide Heteropodatoxin	Cisapride, Ergotoxin, Astemizole, BeKM-1, E-4031	XE-991, Linopirdine, Retigabine
Effects on MP	ChTX depolarized the tissue, increased burst duration and spiking frequency in canine colon ⁷⁸ Not clear on the role of RMP and slow wave potentials in other species	4-AP depolarized, increased the rate of rise of the upstroke potential and prolonged the plateau phase of slow waves in canine colon ^{5,79}	4-AP depolarized ⁷⁹	?	?
Effects on contractility	Increase in contractions in canine colon ¹	TEA (1–5mM) increased contractility ⁸⁰	?	Cisapride induced contraction ^{81–83}	XE991 and Linopirdine increased the integral of tension ⁸⁴
Comments		4-AP effects could be due to inhibition of KA	Hyperpolarization reset K _A current and affects repolarization. KChIP is auxiliary subunit		

* V_a=half activation voltage.

The references given above refer to tissue experiments.

Table 2

Voltage-dependent inward conductances in colonic smooth muscle

	L-type Ca²⁺ Channels	T-type Ca²⁺ Channels	TTX-sensitive Na⁺ Channel	Ca²⁺-activated Cl⁻ Channel
Gene candidate	<i>Cav1.2</i>	<i>Cav3.1, Cav3.2, Cav3.3</i>	<i>Nav1.x</i>	<i>ANO1</i>
IUPHAR name	$\alpha 1C$	$\alpha 1G, \alpha 1H, \alpha 1I$	R-x	Tmem16a
Other names	DHP-sensitive Ca ²⁺ channel	Low voltage-activated Ca ²⁺ channel		CaCC, Anoctamine-1, DOG-1
Expressed cell type	Smooth muscle cell	Smooth muscle cell/ possibly ICC	Smooth muscle cell	Predominantly ICC
Property	High voltage-threshold activation ($V_a^* = -17mV$, $V_h^* = -50mV$), Ca ²⁺ -dependent-inactivation	Low voltage-activation and inactivation ($V_a^* = -45mV$, $V_h^* = -70mV$), fast inactivation (<1ms)	Low voltage-activated, fast inactivation (<1ms)	Ca ²⁺ and voltage-dependent
Unitary conductance	10–30pS	<10pS [*]		0.5pS–9pS
Modulator/Agonist	BayK8644 ACh, Erythromycin FPL64716	ATP, Isoproterenol	Veratridine	Ca ²⁺
Antagonist/Blocker	DHP (Nifedipine, Diltiazem, Nicardipine, Verapamil)	Ni ²⁺ (10–200 μ M), U92032, Mibefradil, Pimozide, Kurotoxin	TTX (2–15nM), Lidocaine, Saxitoxin	Niflumic acid, DIDS, SITS, 9-AC, NPPB
Effects on MP	DHP inhibited the spontaneous spike complexes but not affects RMP ^{85,86}	Involve active repolarization from IJP. Inhibited or reduced the rate of rise on plateau potential in ICC-SM ^{88,89}	?	Niflumic acid hyperpolarized ⁶⁸
Effects on contractility	DHP abolish spontaneous contractions ⁸⁷	?	?	?
Comments		Low voltage-activated inward conductance in SMC reversed at 0mV. It may different from known T-type Ca ²⁺ channel	TTX-resistant Na ⁺ channel (SCN5a, Nav1.5) expressed in canine colonic ICC ⁹⁰	CaCC in colonic ICC may involve STD (see text)

* ; V_a =half activation voltage, V_h =half inactivation voltage.

The references given above refer to tissue experiments.

Table 3

Voltage-independent K⁺ channels in colonic smooth muscle

	Small Conductance Ca ²⁺ -activated K ⁺ Channel	ATP-sensitive K ⁺ Channel	Inwardly Rectifying K ⁺ Channel	G-protein gated Inwardly Rectifying K ⁺ Channel	TWIK-related K ⁺ Channel 1
Gene candidate	<i>KCNN1</i> , <i>KCNN2</i> , <i>KCNN3</i>	<i>KCNJ8</i> , <i>KCNJ11</i>	<i>KCNJ2</i>	<i>KCNJ3</i> , <i>KCNJ6</i>	<i>KCNK2</i>
IUPHAR name	KCa2.1, KCa2.2, KCa2.3	Kir6.1, Kir6.2	Kir2.1	Kir3.1, Kir3.2	K ₂ p2.1
Other names	SK, SKCa	KATP	IRK1	GIRK	TREK-1
Expressed cell type	Smooth muscle cell / PDGFR α ⁺ cell	Smooth muscle cell	ICC	?	Smooth muscle cell
Property	Ca ²⁺ -dependent, Voltage-independent	Weak inward rectification, ADP activation	Strong inward rectification by Mg ²⁺ or polyamine	G $\beta\gamma$ activates GIRK but G α by binding G $\beta\gamma$ inhibit gating	Both leak and voltage dependence depending on s348 phosphorylation
Unitary conductance	5–10pS	17–27pS	10–32pS	30–45pS	90pS
Modulator/Agonist	1-EBIO, DC-EBIO, NS309, cyPPA, SKA-31, β -NAD, ADPribose	NDP, Diazoxide, Pinacidil, Lemakalim, Cromakalim, Nicorandil	PKA phosphorylation, ATP hydrolysis; PIP ₂		Arachidonic acid, Volatile anesthetics, mechanical stretch, PKG
Antagonist/Blocker	Apamin, UCL1684, Bicuculline, Scyllatoxin	ATP, Glibenclamide for associated SUR subunit	Ba ²⁺ , Cs ⁺ , Rb ⁺ , Putrescine	Tertiapin*	L-methionine and derivatives, Ba ²⁺ or Quinidine (mM), PKA, PKC
Effects on MP	Depolarization and inhibition of spontaneous IJP by apamin ^{91,14} β -NAD, ADP ribose hyperpolarize ⁹²	Glibenclamide depolarize, K _{ATP} opener hyperpolarize ^{42,94,95}	Depolarization by Ba ²⁺ 48.97	?	L-methionine depolarize ⁵⁶
Effects on contractility	Increased contractility by apamine ⁹³	glibenclamide and K _{ATP} openers Increase and decrease contractility, respectively ⁹⁶		?	L-methionine increases contractility ⁵⁶
Comments	Fast IJP may be due to activation of SK in FLC (see text)	Associated subunit in colonic smooth muscle is SUR2B		GIRK data only on molecular study in tissue level	Slow IJP may be due to TREK-1 activation (see text)

The references given above refer to tissue experiments.