Calcium-associated mechanisms in gut pacemaker activity

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Abstract

A considerable body of evidence has revealed that interstitial cells of Cajal (ICC), identified with c-Kit-immunoreactivity, act as gut pacemaker cells, with spontaneous Ca2+ activity in ICC as the probable primary mechanism. Namely, intracellular (cytosolic) Ca2+ oscillations in ICC periodically activate plasmalemmal Ca2+-dependent ion channels and thereby generate pacemaker potentials. This review will, thus, focus on Ca2+-associated mechanisms in ICC in the gastrointestinal (GI) tract, including auxiliary organs.

Keywords: interstitial cells of Cajal • calcium oscillations • smooth muscle • voltage-gated Ca2+ channels • non-selective cation channels • transient receptor potential (TRP) homologues • Ca2+-activated Cl− channels • inositol trisphosphate receptors • ryanodine receptors • c-Kit

Introduction

Research has shown that Ca2+-dependent plasmalemmal ion channels are responsible for interstitial cells of Cajal (ICC) pacemaker potentials [1–3], and spontaneous Ca2+ activity in ICC is considered the primary mechanism. Namely, oscillations of the intracellular (cytosolic) Ca2+ concentration ([Ca2+]i) in ICC periodically activate plasmalemmal Ca2+-dependent ion channels, thereby generating pacemaker potentials. This review will, thus, focus on Ca2+-associated mechanisms in ICC in the gastrointestinal (GI) tract including auxiliary organs.

Numerous preparations and methods are used in studies of various types of ICC contained in the GI tract. In the following sections, types of ICC are identified in descriptions of most tissue-level experiments; the term ICC represents interstitial cells expressing c-Kit or other ICC markers in experiments using isolated cells and cultured preparations.

Voltage-gated Ca2+ channels

Voltage-gated Ca2+ channels (VGCC) are thought to play a central role in E–C coupling in smooth muscle
cells, including GI smooth muscle. L-type (high voltage-activated [HVA]) Ca\(^{2+}\) channels appear to be predominant in most smooth muscle cells because dihydropyridine (DHP) Ca\(^{2+}\) antagonists, such as nifedipine and nicardipine largely depress contractile activity. It has been shown that the \(\alpha_1\)-subunit of the smooth muscle L-type Ca\(^{2+}\) channel (Ca\(_{\alpha 1.2b}\)) is a splice variant of the cardiac one (Ca\(_{\alpha 1.2a}\)) and has a higher sensitivity to DHP Ca\(^{2+}\) antagonists [4].

Guinea-pig stomach smooth muscle, frequently used to investigate mechanisms underlying spontaneous electrical activity, referred to as slow waves [5], provides a good example with which we can assess the role of VGCC in smooth muscle tissues showing spontaneous phasic contractions. DHP Ca\(^{2+}\) antagonists completely abolish spontaneous contractile activity, with little effect, however, on pacemaker potentials (and electrical activity recorded from smooth muscle cells, that is, slow waves) [6, 7]. Similar spontaneous electrical activities resistant to the DHP-Ca\(^{2+}\) antagonist have been reported in several other GI smooth muscle tissues [8, 9]. It is thus considered that DHP-sensitive L-type Ca\(^{2+}\) channels play an essential role in E–C coupling in GI smooth muscle cells, although these channels are not involved in the generation of pacemaker electrical activity in ICC (Fig. 1). For this reason, DHP-Ca\(^{2+}\) antagonists are frequently used to differentiate pacemaker electrical activity by suppressing smooth muscle activity. However, pacemaker cells in some tissues, for example, sub-mucosal ICC (ICC-SM) in the colon, produce different responses to DHP Ca\(^{2+}\) antagonists: 1 µM nifedipine completely abolishes the spontaneous plateau potentials [10]. Furthermore, in the guinea-pig stomach, a small inhibitory effect was observed when nifedipine was greater than 10 µM [7].

In cardiac pacemaker cells, T-type (low voltage-activated [LVA]) Ca\(^{2+}\) channels, known to play an important role in pacemaking, are suppressed with low concentrations (~40 µM) of Ni\(^{2+}\) [11]. Applications of similar concentrations of Ni\(^{2+}\) to guinea-pig stomach smooth muscle tissue (including the smooth muscle layer and myenteric plexus) have little effect on spontaneous electrical activity [7]. On the other hand, in the isolated circular smooth muscle layer, which does not contain myenteric ICC (ICC-MY) but contains only intramuscular ICC (ICC-IM), very low concentrations of Ni\(^{2+}\) (1–10 µM) significantly suppress spontaneous electrical activity. The inhibitory effect is more potent in the plateau phase than in the initial upstroke of pacemaker electrical activity, agreeing well with the notion that ICC-IM produces re-generative potentials forming the plateau phase [12, 13]. However, the discrepancy in the Ni\(^{2+}\) concentration range may suggest the existence of Ni\(^{2+}\)-sensitive mechanisms other than T-type Ca\(^{2+}\) channels involved in ICC-IM. Recent reverse transcriptase-polymerase chain reaction (RT-PCR) examinations have provided supporting evidence that neither the L- nor the T-type Ca\(^{2+}\) channel gene was detected in ICC-DMP (deep muscular plexus) and ICC-IM of the murine and human small intestine [14].

The existence of VGCC may differ depending on the ICC types, locations of the gut and species. Ward and Sanders [15, 16] reported that 40 µM Ni\(^{2+}\) cause sizeable reduction (>50%) in the upstroke velocity of spontaneous electrical activity in canine colon, and a combined application of nifedipine (1 µM) and Ni\(^{2+}\) (40 µM) abolishes it. These results suggest a major contribution of T-type Ca\(^{2+}\) channels. In ICC-SM of the murine colon, Hotta et al. [17] reported that the application of either mibebradil (3 µM) or Ni\(^{2+}\) (100 µM) significantly reduced the rate of rise in the upstroke of pacemaker potentials. Kim et al. [18] recorded VGCC currents either sensitive or resistant to DHP Ca\(^{2+}\) antagonists from ICC in the murine colon and small intestine. The DHP-resistant component of VGCC current is blocked by a higher concentration (100 µM) of Ni\(^{2+}\) or by a T-type Ca\(^{2+}\) channel antagonist, mibebradil. The inhibitory effect of mibebradil may involve the blockade of voltage-gated Na\(^{+}\) channels (VGSC) resistant to tetrodotoxin (TTX) (Na\(_{\alpha 1.5}\) encoded by SCN5A), which has been shown to exist in ICC [19, 20].

Non-selective cation channels

Non-selective cation channels (NSCC) can carry an electric charge for ICC pacemaking current, and many of these channels can permeate Ca\(^{2+}\). It is well-known that spontaneous electrical activities in GI smooth muscle tissues require extracellular Ca\(^{2+}\) [5]. Therefore, NSCC may make a significant contribution to ICC pacemaking.

Under a voltage clamp condition, Thomsen et al. [21] and Koh et al. [22] recorded oscillating inward currents from cultured ICC of the murine small intestine. Removal of extracellular Na\(^{+}\) abolishes the oscillatory inward currents [22], suggesting that oscillating...
Inward currents are produced by the periodic activation of NSCC. Nakayama and Torihashi [23] showed that high concentrations (100–120 µM) of Cd²⁺ and Ni²⁺ suppress oscillatory inward currents in cell clusters prepared from the murine small intestine, which contains ICC. Using a special thin muscle layer preparation made by enzymatic treatment under hydrostatic pressure, Goto et al. [24] demonstrated that depolarization steps can evoke large inward currents through NSCC in ICC showing spontaneous electrical activity.

Transient receptor potential (TRP) homologues form NSCC. Epperson et al. [25] and Liu et al. [26] detected mRNA of classical (or canonical) TRP (TRPC), such as TRPC2, TRPC4 and TRPC6 in ICC, using RT-PCR. Torihashi et al. [27] showed immunohistochemical evidence for the expression of TRPC4 in the caveolae where numerous cellular signals interact. Walker et al. [3] recorded oscillatory inward currents similar to TRPC4: a NSCC inward current inhibited by Ca²⁺ (see also Note added in proof).

Melastatin-type TRP (TRPM) homologues are channel/enzyme fusion proteins. TRPM6 and TRPM7 (formerly referred to as <TRPC>), which contain a kinase domain in the C-terminus, are well-known to act as Mg²⁺-permeable channels [28–30]. Kim et al. [31, 32] showed mRNA of TRPM2, TRPM4, TRPM7 and TRPM8 in cultured ICC from the murine small intestine, and they reported that TRPM7 channels play an essential role in generating oscillatory currents in ICC; that is, the ionic selectivity and pharmacological properties are essentially the same between TRPM7 and ICC oscillatory currents. The authors also showed that the knockdown of TRPM7 by the use of siRNA suppressed spontaneous electrical activity in ICC. However, the reduction of TRPM7 expression may affect ICC pacemaking through intracellular Mg²⁺ homeostasis and cell viability [30, 33]. The regulation of intracellular Mg²⁺ via TRPM-like Mg²⁺-permeable channels has been shown in intestinal [34, 35] and vascular smooth muscle cells [36–38].

The frequency and duration of GI pacemaker activity are largely modulated by temperature and energy metabolism [23, 39, 40]. Nakamura et al. [41] suggested that in such modulations of pacemaker activity, several pathways are operating in parallel. Although the mRNA expression of TRPM4 and TRPM8 has been shown in cultured murine ICC [31, 32], the existence of vanilloid type (TRPV) and ankyrin-like TRP (TRPA) channels has not yet been assessed. TRPV1, TRPV2, TRPV3, TRPV4, TRPM2, TRPM4 and TRPM5 are heat activated, whereas TRPM8 and TRPA1 are cold activated [42]. Further investigation into TRP homologue channels may, therefore, clarify the mechanisms underlying the characteristic features of GI pacemaker activity. In addition, mitochondria [43] and sulfonylurea receptors (SUR) [44, 45] may also contribute to the temperature- and energy-dependence of ICC pacemaking.

### Cl⁻ channels

Many reports have suggested that Cl⁻ channels carry pacemaker current in ICC. Spontaneous [Ca²⁺]; oscillations would periodically activate Ca²⁺-activated Cl⁻ channels if such channels exist in ICC. In guinea-pig stomach, Dickens et al. [6] demonstrated that ICC-MY can generate giant pacemaker potentials of ~50 mV in amplitude, reaching ~20 mV in the plateau phase. This potential is close to the equilibrium potential of Cl⁻ (E_Cl = −24 mV) estimated from the intracellular Cl⁻ concentration ([Cl⁻]i: 42 mM) measured in vas deference smooth muscle cells (i.e., [Cl⁻]o: 103 mM) [46, 47]. Measurements of [Cl⁻]i in ICC will provide valuable information for the debate over whether Cl⁻ channels or NSCC are responsible for the pacemaker current.

In early ICC research, Tokutomi et al. [1] recorded oscillatory inward currents in ICC (= c-Kit-positive interstitial cells) isolated from the murine small intestine. This current showed Ca²⁺-dependence and was sensitive to a Cl⁻ channel blocker, 4-acetoamido-4-isothiocyanat-ostilbene-2, 2'-disulfonic acid (SITS). Since then, there has been a growing body of evidence that ICC electrical activity is suppressed by other Cl⁻ channel blockers, such as 4, 4'-diisothiocyanostilbene-2, 2'-disulfonic acid (DIDS) and anthracene-9-carboxylic acid (9-AC) [9, 20, 48, 49]. Huizinga et al. [2] demonstrated the existence of large-conductance (maxi) chloride channels at the single-channel level of patch-clamp recording. On the other hand, Koh et al. [50] reported that a Cl⁻ channel blocker, niflumic acid, blocks NSCC in ICC.

The existence of small conductance Ca²⁺-activated K⁺ channels (SK3 and SK4) has also been shown in ICC of the rat GI tract [51]. In each [Ca²⁺]; oscillation cycle, these channels may contribute to the repolarization phase of the pacemaker potential.
[Ca\textsuperscript{2+}]\textsubscript{i} oscillations

[Ca\textsuperscript{2+}]\textsubscript{i} oscillations in ICC are thought to be a primary mechanism for the generation of pacemaker potentials, which may account for characteristic features of GI pacemaker activity, such as the low voltage sensitivity of the frequency. Publicover et al. [52] reported [Ca\textsuperscript{2+}]\textsubscript{i} oscillations in freshly dispersed and cultured ICC-like cells from canine colon, although c-Kit-immunoreactivity was unidentified. DHP Ca\textsuperscript{2+} antagonists suppress [Ca\textsuperscript{2+}]\textsubscript{i} oscillations in these ICC-like cells. Yamazawa and Iino [53] recorded [Ca\textsuperscript{2+}]\textsubscript{i} oscillations resistant to DHP Ca\textsuperscript{2+} antagonists in ICC of the murine small intestine. No such spontaneous [Ca\textsuperscript{2+}]\textsubscript{i} activity was observed in W/W\textsuperscript{v} mice lacking ICC.

Using cell cluster preparations from the murine small intestine, Torihashi et al. [27] and Nakayama et al. [23] recorded [Ca\textsuperscript{2+}]\textsubscript{i} oscillations synchronized with spontaneous electrical and mechanical activities (Fig. 2). These results agree well with the hypothesis that [Ca\textsuperscript{2+}]\textsubscript{i} oscillations in ICC generate pacemaker electrical activity by periodically activating Ca\textsuperscript{2+}-activated ion channels in the plasma membrane (Scenario 1 in Fig. 3). Moreover, in guinea-pig and mouse stomach ICC, [Ca\textsuperscript{2+}]\textsubscript{i} oscillations appear to be associated with spontaneous electrical and mechanical activities [26, 54–56].

Using high time-resolution Ca\textsuperscript{2+} measurements, Park et al. [57] recently showed that the rising phase of [Ca\textsuperscript{2+}]\textsubscript{i} oscillation in ICC follows the upstroke of the electrical activity recorded from a near-by cell, with a short delay (~60 ms). VGCC insensitive to DHP or VGSC [18–20] may be responsible for the depolarization preceding the [Ca\textsuperscript{2+}]\textsubscript{i} rise. Localized elevation of [Ca\textsuperscript{2+}]\textsubscript{i} in the vicinity of the plasma membrane may first cause the activation of Ca\textsuperscript{2+}-activated ion channels, resulting in depolarization to trigger VGCC and a subsequent global increase in [Ca\textsuperscript{2+}]\textsubscript{i} (Scenario 2 in Fig. 3). Further investigation is necessary to elucidate the details of mechanisms that link [Ca\textsuperscript{2+}]\textsubscript{i} oscillations and pacemaker potentials and to comprehensively address the cell-to-cell coupling among pacemaker cells and smooth muscle cells [58, 59].

Ca\textsuperscript{2+} influx from the extracellular space appears to play an essential role in ICC pacemaker [Ca\textsuperscript{2+}]\textsubscript{i} activity because the removal of extracellular Ca\textsuperscript{2+} abolishes it [27]. NSCC may act as this Ca\textsuperscript{2+} pathway. Applications of Ni\textsuperscript{2+} and Co\textsuperscript{2+} (>100 µM), which competitively block NSCC, suppress [Ca\textsuperscript{2+}]\textsubscript{i} oscillations in ICC [27, 57, 60]. Furthermore, SK&F96365 suppresses or terminates [Ca\textsuperscript{2+}]\textsubscript{i} oscillations [26, 27]. SK&F96365 is often used to suppress Ca\textsuperscript{2+} influx pathways from the extracellular space, including TRP homologue channels, but does not block DHP-sensitive VGCC [61].

ICC can express numerous receptors of neurotransmitters: purinoceptors, neurokinin receptors, muscarinic receptors and prostaglandin receptors [14, 63–66]. Furuzono et al. [67] showed dual effects of ATP and analogues on [Ca\textsuperscript{2+}]\textsubscript{i} oscillations in ICC of the murine small intestine: excitation in the presence of TTX and inhibition in the absence of TTX. It is suggested that NO released from nitrergic nerves via activation of purinoceptors suppresses [Ca\textsuperscript{2+}]\textsubscript{i} oscillations in ICC, presumably through a cGMP signalling pathway, while in the presence of TTX, a [Ca\textsuperscript{2+}]\textsubscript{i} transient is evoked through the activation of purinoceptors on the surface of ICC.

Intracellular Ca\textsuperscript{2+} release channels

Although Ca\textsuperscript{2+} influx from the extracellular space is likely essential to maintain [Ca\textsuperscript{2+}]\textsubscript{i} oscillations in ICC, Ca\textsuperscript{2+} release from intracellular Ca\textsuperscript{2+} stores, that is, endoplasmic reticulum (ER), appears to make a major contribution to the [Ca\textsuperscript{2+}]\textsubscript{i} increase. Suzuki et al. [68] and Takano et al. [69] showed that spontaneous electrical and mechanical activities are greatly impaired in the stomach smooth muscle of mice lacking the type-1 inositol trisphosphate receptor (InsP\textsubscript{3}R1). Liu et al. [26] showed that among three InsP\textsubscript{3}R isoforms InsP\textsubscript{3}R1 and InsP\textsubscript{3}R2 are predominant in ICC in the murine stomach. Aoyama et al. [62] reported that InsP\textsubscript{3}R2 and InsP\textsubscript{3}R3 are predominant in the small intestine. Taken together, these findings suggest that InsP\textsubscript{3}R1 expressed in stomach ICC plays an important role in generating pacemaker activity on its own without using intercellular mechanisms, while the role of InsP\textsubscript{3}R1 may be substituted by InsP\textsubscript{3}R2 and/or InsP\textsubscript{3}R3 in small intestine ICC. Therefore, it is of interest to check whether spontaneous activity is preserved in the small intestine of mutant mice lacking InsP\textsubscript{3}R1.

There is an increasing body of pharmacological evidence for the involvement of InsP\textsubscript{3}R in ICC pacemaker activity. The applications of 2-aminoethoxydiphenyl borate (2-ABP) and xestospongin C (Xe C), membrane-permeable blockers for InsP\textsubscript{3}R, suppress
or terminate ICC electrical and \([\text{Ca}^{2+}]_i\) activities in numerous GI preparations (Table 1) [26, 55, 57, 58, 60, 62, 70–73]. It has also been shown that the application of heparin with a reversible permeabilization loading procedure suppressed depolarization-induced electrical activities reflecting ICC-IM activity [74]. On the other hand, 2-APB affects TRP homologue channels, including TRPM7 [75]. The inhibitory effect of 2-APB on \([\text{Ca}^{2+}]_i\) oscillations might involve the blockage of TRPM7 because this channel reportedly plays an essential role in generating ICC pacemaker activity [31, 32].

Another important \(\text{Ca}^{2+}\) release channel is the ryanodine receptor (RyR). Using cell cluster preparations from the murine small intestine, Aoyama et al. [62] showed that in addition to blockers for \(\text{InsP}_3\text{R}\) and \(\text{Ca}^{2+}\) influx, RyR blockers and FK506, which modulates RyR activity through FK506-binding proteins (FKBP), all suppress ICC pacemaker \([\text{Ca}^{2+}]_i\) oscillations. RT-PCR examinations of ICC revealed a predominant expression of RyR3 and a corresponding expression pattern of FKBP isoforms (expression of both FKBP12 and FKBP12.6). Liu et al. [26, 56] showed essentially the same results in murine stomach ICC. Also, the application of ryanodine significantly suppresses spontaneous \([\text{Ca}^{2+}]_i\) oscillations in ICC-like cells of gut-like organ formed from mouse embryonic stem (ES) cells [76]. These results suggest that the coordination of the two families of \(\text{Ca}^{2+}\) release channels, that is, RyR and \(\text{InsP}_3\text{R}\), produces ICC pacemaker \([\text{Ca}^{2+}]_i\) activity under the support of \(\text{Ca}^{2+}\) influx from the extracellular space. Furthermore, based on this hypothesis, pacemaker-like cells have been produced by genetic manipulations [62]. Namely, HEK293 cells which express little RyR have yielded spontaneous \([\text{Ca}^{2+}]_i\) oscillations.

![Fig. 1 The action sites of drugs on \(\text{Ca}^{2+}\)-associated mechanisms in ICC pacemaker activity. ICC and SM in this figure represent interstitial cells of Cajal and smooth muscle cells, respectively. For other abbreviations, see text.](image_url)
oscillations after the transfection of RyR3. This is also true for RyR2 (Aoyama et al. unpublished observation). Mice lacking RyR3 show apparently normal growth and reproduction [77]. In these mice, RyR2 may compensate for the role of RyR3.

In ICC-like interstitial cells of the rabbit portal vein and urethra, Harhun et al. [78] and Johnston et al. [79], respectively showed essentially the same pharmacological profiles of [Ca^{2+}] oscillations. In generating [Ca^{2+}] oscillations, ICC-like cells in these tissues appear to employ both InsP_{3}R and RyR in addition to Ca^{2+} influx pathways. On the other hand, several studies have reported no significant effect of ryanodine on ICC pacemaker [Ca^{2+}] activity of the GI tract [57, 60, 70, 80].

Gastrointestinal stromal tumours (GIST), the most common mesenchymal tumours of the human GI tract, are thought to derive from ICC by gain-of-function mutations of c-Kit [81]. The application of the selective c-Kit-receptor inhibitor, imatinib mesylate, which is used to treat advanced GIST, suppresses myogenic activity of the human small intestine [82]. Furuzano et al. [83] reported that isolated ICC-like tumour cells from a human duodenal GIST with the most frequent type of gain-of-function mutation only occasionally produced spontaneous [Ca^{2+}] activity. These ICC-like GIST cells expressed InsP_{3}R1 and InsP_{3}R2, but RyR2/3 were below detectable levels (Furuzano et al., unpublished observation). The low expression level of RyR may account for the poor spontaneous [Ca^{2+}] activity in these GIST cells. Evidence is, however, still insufficient to address how intracellular Ca^{2+} release channels contribute to ICC pacemaking. Comprehensive studies, including the link with Ca^{2+} influx pathways and other intracellular Ca^{2+} compartments (e.g. close association of caveolae, ER and mitochondria [84, 85]) are required.

**ICC-like cells in auxiliary digestive organs**

ICC-like cells, that is, c-Kit-positive interstitial cells, exist outside the musculature of the GI tract [86], including the auxiliary organs of the GI tract. Popescu et al. [87] reported the existence of ICC-like cells in the human and rat pancreas. Hinescu et al. [88] and Sun et al. [89] reported ICC-like cells in the human and murine gall bladders, respectively. Lavoie et al. [90] showed spontaneous [Ca^{2+}] activity in ICC-like cells in the guinea-pig gall bladder and suggested a role of generating spontaneous rhythmicity. Furthermore, ICC-like cells exist in the hepatic portal vein [91, 92], which transports nutrient molecules to the liver and is known as a spontaneously active vessel. Harhun et al. [78, 93] showed spontaneous [Ca^{2+}] oscillations associated with membrane depolarizations in ICC-like interstitial cells of the rabbit
Fig. 3 Plausible mechanisms linking spontaneous [Ca\(^{2+}\)]\(_i\) oscillations and pacemaker potentials in ICC.

### Table 1

Inhibitory effects of numerous Ca\(^{2+}\)-related drugs on pacemaking [Ca\(^{2+}\)]\(_i\) activity in ICC and ICC-like interstitial cells. (+) and (-) represent an inhibitory effect and a lack of significant effect, respectively. The numbers in brackets indicate references. Single crosses (†) indicate examinations of drugs on pacemaker potentials, not on [Ca\(^{2+}\)]\(_i\) oscillations.

| Drug Type      | DHP Ca\(^{2+}\) blockers | Mibefradil | SK&F96365 | 2-APB | Xe C | Ryanodine |
|----------------|---------------------------|------------|-----------|-------|------|-----------|
| Murine stomach | (-): [26, 56]             | (+): [26]  | (+): [26] | (+): [26] | (+): [56] |
| Murine small intestine | (-): [27, 53, 57, 62, 67] | (+): [57]  | (+): [27] | (+): [57, 62] | (+): [62, 70] | (+): [62] |
| Murine colon   | (+): [10]†                | (+): [17]† |           |       |      |           |
| Canine colon   | (+): [52]                 |            |           |       |      |           |
| Guinea-pig stomach | (-): [55, 80]             | (+): [55, 58, 80] |       | (-): [80] |
| Human small intestine | (-): [60]                | (+): [60]  | (+): [60] |       | (-): [60] |
| Rabbit portal vein | (-): [78]               | (+): [78]  | (+): [78] | (+): [78] | (+): [78] |
| Rabbit urethra | (-): [79]                | (+): [79]  | (+): [79] | (+): [79] | (+): [79] |
portal vein and also suggested the contribution of both types of intracellular Ca$^{2+}$ release channels, that is, InsP$_3$R and RyR. It is of great interest to examine the similarity and dissimilarity of mechanisms underlying spontaneous [Ca$^{2+}$]$_i$ activities between ICC and ICC-like cells distributed over the entire body. Such studies will provide valuable information for planning systematic therapies and for developing tissue- and organ-specific drugs.

**Note added in proof**

Recently, two research groups reported that spontaneous electrical activity can be still recorded in the GI tract of TRPC4$^{-/-}$ mice (Lee et al., Mol. Cells 2005; 20: 425–31; Sanders et al., Annu. Rev. Physiol. 2006; 68: 307–43). Therapeutically, it is likely that TRPC4 plays a role in generating ICC pacemaker activity in parallel with after Ca$^{2+}$-permeable channels.

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