

Invited Review

The relationship of TRP channels to the pacemaker activity of interstitial cells of Cajal in the gastrointestinal tract

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Abstract

Interstitial cells of Cajal (ICCs) are a fundamental component of the pacemaker apparatus of the gastrointestinal (GI) tract. They have special properties that make them unique in their ability to generate and propagate slow waves in gastrointestinal smooth muscle. The pacemaker current that generates slow waves is initially due to a voltage-independent, Ca²⁺-inhibited, non-selective cationic conductance in ICC. The classical transient receptor potential (TRPC) channel 4 was suggested as a molecular candidate for the nonselective cation channel (NSCC) responsible for the pacemaker activity. We have shown that TRPC4^{-/-} mice display normal slow waves and suggest that TRPC4 might be an essential component of the NSCC activated by muscarinic stimulation. Finally, we suggest that TRPM7 is the molecular candidate for the NSCC responsible for pacemaker activity in ICCs on the basis of electrophysiological, molecular biological, and immunohistochemical experiments.

Key words: transient receptor potential (TRP), pacemaker activity, interstitial cells of Cajal (ICCs), nonselective cation channel (NSCC)

Introduction

Interstitial cells of Cajal (ICCs) generate the electrical pacemaker activity (slow waves) in gastrointestinal (GI) smooth muscle (Ward *et al.*, 1994; Huizinga *et al.*, 1995; Sanders, 1996). Slow waves propagate within ICC networks, conduct into smooth muscle cells via gap junctions, and initiate phasic contractions by activating Ca²⁺ entry through L-type Ca²⁺ channels.

Possible mechanisms for the pacemaker activity in interstitial cells of Cajal

Using cultured ICCs, it has been shown that the pacemaker currents generated by ICCs resulted primarily from the activation of a voltage independent, nonspecific cation conductance

and that they may conduct Na^+ and Ca^{2+} in physiological ionic gradients (Thomsen *et al.*, 1998; Koh *et al.*, 1998). The pacemaker conductance in ICCs was shown to be regulated by intracellular Ca^{2+} handling (Ward *et al.*, 2000). Several investigators have concluded that the release of Ca^{2+} from inositol 1,4,5-trisphosphate (IP_3) receptor-operated stores is the basic cellular event that initiates the pacemaker current. The importance of Ca^{2+} release from IP_3 receptor-operated stores in the generation of slow waves has been demonstrated pharmacologically using cultured ICCs (Ward *et al.*, 2000), in experiments on mutant mice lacking the $\text{IP}_3\text{R1}$ isoform of the IP_3 receptor (Suzuki *et al.*, 2000), and in pharmacological studies of intact muscle strips (Ward *et al.*, 2000; Malysz *et al.*, 2001). These IP_3 receptors are highly expressed by ICCs and are closely associated with the plasma membrane and mitochondria.

Ward *et al.* (2000) suggested that the close association between the IP_3 receptor-dependent Ca^{2+} stores and both the mitochondria and ion channels in the plasma membrane created a basic cellular structure. Ca^{2+} release from Ca^{2+} stores in ICCs drives the initiation of pacemaker currents. Release of Ca^{2+} from IP_3 receptors initiates Ca^{2+} uptake into mitochondria. Mitochondria in ICC experience Ca^{2+} oscillations at the same frequency as the pacemaker currents. The rise in mitochondrial Ca^{2+} was shown to slightly precede the activation of the pacemaker current (Ward *et al.*, 2000), and therefore the pacemaker channels in the plasma membrane are activated by the falling phase of the localized Ca^{2+} transients.

However, the relationship between the mitochondrial calcium oscillation and the intracellular calcium oscillation or pacemaker potentials remains to be determined, considering that single ICC did not demonstrate regular pacemaker potentials and only a cluster of ICCs showed regular pacemaker activity.

Several investigators have studied the ionic conductances involved in pacemaker activity and the relationship between Ca^{2+} release and the activation of the pacemaker current. One report suggested that ICCs from the murine small intestine generated spontaneous Ca^{2+} -activated Cl^- currents (Tokutomi *et al.*, 1995). This conclusion was based on pharmacological tests using a Cl^- channel-blocking drug (4-acetoamido-4-isothiocyanat-ostilbene-2,2-disulphonic acid; SITS). From studies of intact muscle preparations, other investigators have suggested that Ca^{2+} -activated Cl^- conductance is responsible for the pacemaker currents in ICCs (Hirst *et al.*, 2002; Kito *et al.*, 2002). These investigators have suggested that Ca^{2+} released from IP_3 receptor-operated stores directly activates Ca^{2+} -activated Cl^- channels in the plasma membrane. Also two additional papers have reported that both high conductance Cl^- channels and inwardly rectifying Cl^- channels participate in the pacemaker activity of ICCs (Huizinga *et al.*, 2002; Zhu *et al.*, 2005). A high-conductance chloride channel was spontaneously and rhythmically active at the same frequency as the rhythmic inward currents in ICC pacemaker activity and at the single channel level, while chloride channels were seen to be associated with the generation of the rhythmic changes in the membrane potential. In whole cell patch-clamp studies, ICCs expressed an inwardly rectifying Cl^- current that was not sensitive to changes in the cation composition of the extracellular solution.

Another group has suggested that Ca^{2+} -inhibited, non-selective cation channels are periodically activated at the same frequency as the pacemaker currents in murine ICCs (Koh *et*

al., 2002). Reducing the cytoplasmic Ca^{2+} activated inward currents and single-channel currents in the entire cell. When patch-clamping, the ICCs were found to contain nonselective cation channels with a single channel conductance of 13.5 pS. These channels were inhibited in a concentration-dependent manner by Ca^{2+} applied to the intracellular surface of the membrane. In addition, the presence of a nonselective cation conductance has been reported in freshly dispersed ICCs from the murine small intestine (Goto *et al.*, 2004). The c-kit-positive CD34 negative ICCs in enzymatically treated intestinal muscle showed spontaneous and rhythmic potential fluctuations, and a large transient inward current was evoked by depolarization under voltage clamp conditions. Once the inward current was triggered, it took a regenerative time course and lasted approximately 500 ms. This inward current showed a reversal potential of around +3 mV and was considered to be due to non-selective cation channels.

TRPC4 is an essential component of the nonselective cation channel activated by muscarinic stimulation in the murine stomach

After the transient receptor potential (TRP) gene was found, many researchers suggested that the TRP protein might be the candidate for the NSCC recorded in many tissues. TRP channels were first cloned from *Drosophila* species and constitute a superfamily of proteins that encode a diverse group of Ca^{2+} -permeable NSCCs (Clapham, 2003). The TRP family is divided into 3 subfamilies: classic (TRPC), vanilloid type (TRPV), and melastatin type (TRPM) (Clapham, 2003). TRPC channels mediate cation entry in response to phospholipase C activation, whereas TRPV proteins respond to physical and chemical stimuli, such as changes of temperature, pH, and mechanical stress (Clapham, 2003). The TRPM subfamily members differ significantly from other TRP subfamilies in terms of domain structure, cation selectivity, and activation mechanisms (Clapham, 2003).

TRPC4 was suggested as a molecular candidate for the Ca^{2+} -inhibited, nonselective cation conductance in ICCs (Walker *et al.*, 2002). In ICCs, calmidazolium and W-7 (both calmodulin inhibitors) increased the activity of the 13.5 pS nonselective cation channels under both on-cell and off-cell recording conditions, suggesting that inhibition of channel gating occurs via Ca^{2+} /calmodulin binding. Expressed TRPC4 channels display properties similar to that of the native pacemaker conductance, such as similar single channel conductance, negative regulation by Ca^{2+} , and activation by calmodulin inhibitors. But, TRPC4 was activated by stimulating phospholipase C but not by Ca^{2+} -store depletion or decreases in intracellular calcium concentration (Zhu *et al.*, 2003; Lee *et al.*, 2003). These two research groups argued that TRPC4 is activated by Ca^{2+} -store depletion or a decrease of intracellular calcium concentration. In addition, the electrophysiological properties of expressed TRPC4 were found to be different in two laboratories. Freichel *et al.* (2001) showed an inwardly rectifying current-voltage relationship for TRPC4, whereas Walker *et al.* (2002) showed an outwardly rectifying current-voltage relationship. On the other hand, Torihashi *et al.* (2002) showed that only TRPC4 exists in ICCs using anti-c-kit and anti-TRPC4 antibodies.

We have shown that pacemaker activity appears not to be dependent upon the expression of TRPC4 channels (Lee *et al.*, 2005). TRPC4^{-/-} mice displayed normal slow waves. Instead of its

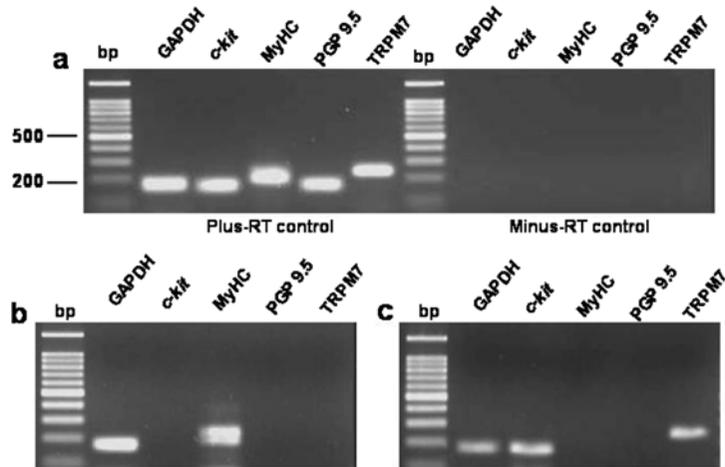


Fig. 1. RT-PCR detection of TRPM7 in single ICCs. Gel electrophoresis of the RT-PCR products from the mouse colon (a), an isolated single intestinal myocyte (b) and a single ICC (c). RT-PCR products were resolved on 2% agarose gel and visualized by ethidium bromide staining. Size markers were used to indicate the size of the experimental fragments (Lane 1). (a) RT-PCR detection of glyceraldehyde phosphate dehydrogenase (GAPDH; house keeping; 170 base pairs [bp]), c-kit (ICC marker; 162 bp), myosin heavy chain (MyHC; smooth muscle marker; 234 bp), protein gene product 9.5 (PGP 9.5, a pan-neural marker; 169 bp), and TRPM7 (207 bp) in mRNAs from mouse colon tissue. Left, Plus-RT (Reverse Transcriptase) control; right, Minus-RT control. (b) Single intestinal myocyte shows RT-PCR fragments for MyHC (234 bp) but not for TRPM7. (c) Single ICC shows RT-PCR fragments for both c-kit (162 bp) and TRPM7 mRNA. From Kim *et al.* (2005) with permission.

role in pacemaker activity, TRPC4 seems to be a molecular component of the nonselective cation channel that is activated by muscarinic stimulation. Muscarinic stimulation can activate NSCC in TRPC4^{+/+} mice but not in TRPC4^{-/-} mice. TRPC4 seems to be involved in GI excitation by muscarinic stimulation of ICCs.

TRPM7 as a candidate for the NSCC responsible for pacemaker activity in ICCs

After identifying the role of TRPC4, we searched for another molecular candidate for the NSCC involved in the pacemaker activity of ICCs.

We have suggested that TRPM7 is required for pacemaker activity in ICCs (Kim *et al.*, 2005). The characteristics of TRPM7 are as follows: (1) strong outward rectification of the current-voltage relationship; (2) the same divalent cation conductance sequence of the TRPM7-like current ($Zn^{2+} > Ni^{2+} > Ba^{2+} > Co^{2+} > Mn^{2+} > Sr^{2+} > Ca^{2+}$) and the same monovalent cation conductance sequence ($Cs^{+} > Li^{+} > Na^{+}$); (3) modulation by intracellular and extracellular Mg^{2+} ; (4) inhibition by external 2-APB, spermine, SKF96365, quinidine, La^{3+} , and Gd^{3+} ; (5) identification of TRPM7-like immunoreactivity in c-kit-positive ICCs; (6) the existence of TRPM7 mRNA in single ICC but not in single smooth muscle cells (Fig. 1); and (7) inhibition of pacemaking activity with RNAi of TRPM7.

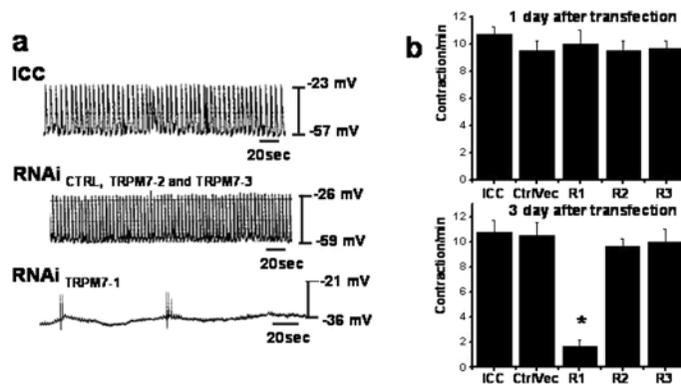


Fig. 2. RNAi effects in clusters of ICCs. (A) Under current clamp conditions, RNAi_{CTRL}, RNAi_{TRPM7-2}, and RNAi_{TRPM7-3} show no change in pacemaker activity compared with the original activity, but RNAi_{TRPM7-1} decreased the pacemaker activity from 16 ± 2 cycles per minute in the original to 1.6 cycles per minute. (B) Population data for the manipulations are expressed as contraction frequency. Values are mean \pm SEM. * $P < .05$. From Kim *et al.* (2005) with permission.

In our recent paper (Kim *et al.*, 2005) we reported making three siRNA for TRPM7 as follows: RNAi_{TRPM7-1}, RNAi_{TRPM7-2} and RNAi_{TRPM7-3}. Under current clamp conditions ($I = 0$), RNAi_{TRPM7-1} decreased the frequency of pacemaker activity to 1.6 cycles per minute, whereas the RNAi control vector, RNAi_{TRPM7-2}, and RNAi_{TRPM7-3} produced no changes in pacemaker activity (Fig. 2). The loss of TRPM7 in ICCs is responsible for reducing both pacemaker activity and contraction frequency.

Initially, a Ca^{2+} inhibited, nonselective cation conductance contributes to the pacemaker current that initiates slow wave activity (Koh *et al.*, 1998). TRPC4 (Walker *et al.*, 2002) and TRPV6 (Yue *et al.*, 2001) were suggested as molecular candidates for a Ca^{2+} -inhibited, nonselective cation conductance channel. In TRPC4 knockout mice, we could still record slow waves (Lee *et al.*, 2005), so TRPV6 was initially suggested as a molecular candidate for CRAC (Ca^{2+} release activated Ca^{2+}) channels (Yue *et al.*, 2001). However, subsequent studies showed contradictory results, and further studies are needed for a concrete conclusion. In our experiments, ruthenium red did not inhibit pacemaker activity in cultured ICC clusters; which suggests that TRPV5 and TRPV6 are at least not involved in pacemaker activity, although ruthenium red blocks other currents.

Closing remarks

ICCs generate the electrical pacemaker activity (slow wave) in GI muscle. Slow waves propagate within ICC networks, conduct into smooth muscle cells via gap junctions, and initiate phasic contractions (gut motility) by activating Ca^{2+} entry through L-type Ca^{2+} channels. Although neurotransmitters, hormones, or inflammatory cytokines can modulate pacemaker activity, the generation of the pacemaker current in ICCs is an essential event leading to GI motility. Consequently, ICCs are involved not only in physiological GI motility, but also in many

bowel disorders, including inflammatory bowel disease, chronic idiopathic intestinal pseudo-obstruction, intestinal obstruction with hypertrophy, achalasia, Hirschsprung's disease, juvenile pyloric stenosis, juvenile intestinal obstruction, and anorectal malformation.

We have suggested that as a primary molecular candidate for the NSCC responsible for pacemaker activity in ICCs, TRPM7 must have an important role in gut motility. Further investigations about the intracellular mechanisms in ICCs relating to TRPM7 are necessary to gain a more complete understanding of the mechanisms involved in the pacemaker activity of ICCs.

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