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Unraveling Smooth Muscle Contraction: The TRP Link

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Parasympathetic signaling via muscarinic receptors regulates gastrointestinal smooth muscle function. Stimulation of muscarinic receptors leads to the activation of mI_{CAT} , which is central and essential in the regulation of smooth muscle contraction. The identity of the components of this critical channel has now been established in a study reported in this issue of *GASTROENTEROLOGY* by Tsvilovsky et al.¹ This study clarifies the relative contributions of M2 and M3 receptors, as well as their respective mechanisms, in the regulation of smooth muscle contraction. The findings are potentially useful for designing clinical strategies utilizing TRP channel modulators for treating intestinal and gastric disorders.

The muscarinic receptors play a key role in the parasympathetic nervous control of various peripheral tissues including smooth muscles.^{2,3} The neurotransmitter acetylcholine, acting via muscarinic receptors, controls excitation–contraction coupling in gastrointestinal smooth muscle cells.³ Pharmacologic studies of gastrointestinal smooth muscle contraction in response to acetylcholine and other muscarinic agonists have suggested that M3 as well as M2 muscarinic receptors are involved in this process. While the M2 receptor is coupled functionally to pertussis toxin (PTX)-sensitive $G_{i/o}$ proteins, which when activated inhibit adenylyl cyclases, the M3 receptor is coupled to PTX-insensitive $G_{q/11}$ proteins that stimulate phospholipase C β (PLC β).^{3,4} The latter leads to breakdown of phosphoinositides with production of inositol-1,4,5-trisphosphate (IP₃) and diacylglycerol (DAG). This signaling leads to an increase in intracellular $[Ca^{2+}]$ as a result of the release of Ca^{2+} from intracellular Ca^{2+} stores as well as activation of Ca^{2+} entry. Intracellular Ca^{2+} release is triggered primarily by the binding of IP₃ to IP₃ receptors, whereas Ca^{2+} entry can potentially be mediated via several different mechanisms such as store-operated or receptor-operated channels. Store-operated Ca^{2+} entry is stimulated by the depletion of Ca^{2+} in the intracellular Ca^{2+} stores. This type of mechanism has been suggested to have a role in the function of some smooth muscle cells.^{5,6} Other cation channels are regulated by DAG or PIP₂ and thus depend on PIP₂ per se or its hydrolysis. These Ca^{2+} entry pathways have also been associated with smooth muscle contraction.⁶

In various gastrointestinal smooth muscles, muscarinic receptor stimulation results in the generation of a nonselective cation current referred to as muscarinic-activated cation current (mI_{Cat}).^{3,4,7} Intriguingly, the activation of mI_{Cat} requires costimulation of both M2 and M3

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Conflicts of interest

The author discloses no conflicts.

receptor subtypes.⁷ Under physiologic ionic conditions this current, primarily carried by Na^+ , causes depolarization of the cell resulting in activation of voltage-dependent Ca^{2+} channels.^{2,3} The net effect of these events is an increase in cytosolic $[\text{Ca}^{2+}]$ that initiates contraction. Lack of voltage-gated calcium channel activity impairs smooth muscle function, illustrating the absolute requirement of this channel.^{2,3,8} However, under physiologic conditions, the activation of voltage-dependent Ca^{2+} channels is critically dependent on the membrane potential change induced by mI_{Cat} . Thus, cationic channel activation in response to muscarinic receptor stimulation and influx of Na^+ , and perhaps Ca^{2+} as well, via this channel is suggested to be the primary and most important mechanism underlying the contractile response to cholinergic stimulation. However, the signal transduction pathway(s) underlying mI_{Cat} and the molecular identity of the channel(s) mediating this current have not yet been determined.

Members of the transient receptor potential canonical (TRPC) family have been receiving increasing attention as molecular candidates for nonselective cationic channels activated by G-protein–coupled receptors in various tissues, including smooth muscles.^{6,9} TRPCs form Ca^{2+} -permeable cation channels that are activated in response to stimulation of plasma membrane receptors that lead to hydrolysis of PIP_2 . As discussed, both receptor-operated and store-operated Ca^{2+} entry mechanisms can be activated in response to this type of signaling. Studies reported over the past 10 years have suggested that TRPCs can contribute toward both types of Ca^{2+} entry channels.^{6,9} It is important to note that although the role for TRPC channels in SOCE is still a matter of controversy, data demonstrating that TRPCs mediate receptor-operated Ca^{2+} entry pathways are quite conclusive. Various TRPC channels are expressed in different types of vascular and gastrointestinal smooth muscle cells. TRPC6 expression in HEK293 cells reproduced almost exactly the essential biophysical and pharmacologic properties of cationic channels activated via α_1 -adrenoceptors in rabbit portal vein, suggesting TRPC6 as a possible molecular candidate for this channel.¹⁰ Although studies with TRPC6^{-/-} mice showed that TRPC6 has a distinct, nonredundant role in smooth muscle vascular tone,¹¹ because of a compensatory effect mediated by up-regulation of TRPC3 in these cells, the conclusive role of TRPC6 was not established. In a later study, TRPC6 was shown to have a unique and indispensable role in acute hypoxic pulmonary vasoconstriction.¹² Among the detected TRPC channels, TRPC1 and TRPC4 have been reported to mediate SOCE in smooth muscle cells and thereby influence contraction. However, pressure-induced constriction of cerebral arteries was not impaired in TRPC1^{-/-} mice and smooth muscle cells from cerebral arteries activated by hypo-osmotic swelling and positive pipette pressure showed no significant differences in cation currents compared with wild-type cells.¹³ Moreover, smooth muscle cells of TRPC1^{-/-} mice isolated from thoracic aortas and cerebral arteries showed no change in store-operated Ca^{2+} entry.¹⁴

TRPC channels have also been individually expressed in HEK293 cells and tested for their ability to generate currents similar to mI_{Cat} of the gastric myocyte. Although functional comparison demonstrated that TRPC5 current exhibits an $I-V$ relationship and relative ion selectivity similar to that of native mI_{Cat} ,¹³ possible contribution of TRPC5 to intestinal smooth muscle contraction was not supported by expression data, which showed that the channel was not deleted in these tissues. However, similarities in the properties of

heterologously expressed TRPC4 and native currents in intestinal smooth muscle suggested that this isoform may play a major role in the receptor-operated cation current mI_{CAT} .^{15,16} Importantly, the activation of TRPC4 requires PTX-sensitive G proteins, a rise in $[Ca^{2+}]_i$, as well as breakdown of PIP₂ by PLC β .¹⁷ These are also properties of mI_{CAT} in native ileal smooth muscle cells. Despite these similarities and the considerable data demonstrating the properties of the TRPC4-mediated cation current, conclusive demonstration of the involvement of TRPC isoforms in gastrointestinal smooth muscle cell function has been lacking.

An elegant study reported by Tsvilovskyy et al¹⁸ unravels the mystery associated with mI_{CAT} in intestinal smooth muscle cells. In this report, the authors present conclusive evidence that demonstrates that TRPC4 and TRPC6 function as 2 separate channels responsible for mI_{CAT} . Their findings suggest that TRPC4 and TRPC6 couple muscarinic receptors to depolarization of intestinal smooth muscle cells, voltage-activated Ca^{2+} influx and muscle contraction, consequently regulating small intestinal motility in vivo. Taken together, these findings finally establish the identity of the critical cation channels associated with mI_{CAT} , thus elucidating a mechanism that was first described almost a quarter of a century ago (Bolton, Casteels, Droogmans, van Breemen). Tsvilovskyy et al¹ have studied mI_{CAT} activation and smooth muscle function in mice lacking TRPC4 alone, TRPC6 alone, or both TRPC4 and TRPC6 together. Their data show that in intestinal smooth muscle cells TRPC4 forms a 55-pS cation channel that contributes >80% of mI_{CAT} and TRPC6 the remaining <20%. There appeared to be no overlap or compensation between the currents mediated by TRPC4 and TRPC6 in the single TRPC knockouts while mI_{CAT} was completely eliminated in the TRPC4/TRPC6 double knockouts. Furthermore, TRPC4-deficient ileal myocytes displayed greatly diminished carbachol-induced membrane depolarizations as well as atropine-sensitive contraction elicited by acetylcholine release from excitatory nerve input. Simultaneous deletion of TRPC6 aggravated these effects. By contrast, resting membrane potential and spontaneous contractile activity of longitudinal smooth muscle fibers were not affected by the lack of TRPC4 and TRPC6; only muscarinic receptor-induced depolarizations and contractile responses were significantly reduced. Direct physiologic consequences were examined by assessing intestinal motility and clearance, both of which were reduced substantially in mice lacking TRPC4 and TRPC6. The findings in this paper demonstrate for the first time that TRPC4 and TRPC6 constitute the muscarinic receptor-activated channels in gastrointestinal smooth muscle cells and thereby critically regulate smooth muscle contraction (Figure 1).

A key finding of this study is that the Ca^{2+} -dependent regulation of TRPC4 which includes facilitation at lower but inhibition at higher Ca^{2+} concentration can explain the oscillations of carbachol-induced membrane potential changes observed in wild-type cells,¹⁸ but not in TRPC4-deficient myocytes. The TRPC4 channel can also account for the dependence of mI_{CAT} on M2 muscarinic receptor as its activation involves PTX-sensitive $G_{i/o}$ proteins.¹⁷ Moreover, 2 isoforms of TRPC4, TRPC4 α and TRPC4 β , are present in the intestinal smooth muscle cells. Previously, it has been established that only TRPC4 α , but not TRPC4 β , is regulated by PIP₂.¹⁷ However, because TRPC4 α and TRPC4 β coassemble in the same channel complex, the major mI_{CAT} should also be subject to PIP₂ regulation just like TRPC4 α , and this is indeed the case.¹⁸ Therefore, TRPC4, the major contributor of mI_{CAT} ,

seems to be the channel that requires costimulation of both M2 and M3 signaling pathways. On the other hand, TRPC6, which has a smaller contribution to mI_{CAT} (about 20%), is primarily activated via coupling to the M3 muscarinic receptor, which stimulates PLC- β and produces DAG.^{19,20} In the case of intestinal smooth muscle, the 2 channels seem to function independently but exert concurrent nonredundant effects on the tissue.

Other channels, including members of the TRP family, have been implicated in various other functions of smooth muscles, including proliferation, vasorelaxation, stress response, and stretch.^{6,9} Some of these, for example, store-dependent cation channels, can be expected to be activated in response to muscarinic receptor stimulation of cells. Notably, TRPC4 has been reported as an essential component of these channels in vascular endothelial cells.²⁰ The contribution of store-operated calcium entry to gastrointestinal smooth muscle contraction as well as the role of TRPC4 in this process cannot be excluded based on the current data and needs to be further evaluated. Another related issue is the distinct biophysical properties of TRPC4. TRPC4 forms calcium selective store-operated channels in endothelial cells, although it seems to be a component of nonselective cation channels in intestinal smooth muscle cells. The basis for this functional diversity represents an interesting problem. Future studies should resolve whether this is because of differences in channel composition or tissue-specific regulation of the channel. Gating versatility is an emerging feature of TRP channels that seems to be an important determinant of their role as physiologic signal integrators.

In conclusion, muscarinic receptors play a key role in the parasympathetic nervous control of gastrointestinal tract. The neurotransmitter acetylcholine stimulates muscarinic receptors, M2 and M3, leading to the activation of cationic channel(s) and generation of mI_{CAT} , which depolarizes the cells providing the critical trigger for stimulation of voltage-dependent Ca^{2+} channels. The ensuing influx of Ca^{2+} is essential for contraction and intestinal motility. The exact molecular identity of the critical channels involved in generating mI_{CAT} in gastrointestinal cells has now been determined by Tsvilovsky et al, who show that mI_{CAT} is actually composed of multiple channel activities mediated by TRPC4 and TRPC6 (Figure 1). These findings reveal TRPC4 and TRPC6 as the missing links in our understanding of the mechanisms involved in cholinergic contraction; namely, the components of the channel(s) for mI_{CAT} and how M2 and M3 muscarinic receptors converge to regulate the contraction. More important, based on this study, TRPC4 and TRPC6 are potentially important clinical targets that can be utilized for modulating smooth muscle function. Of course, further work is required to demonstrate the relevance of these channels in smooth muscle cells from other tissues and species and also how they are affected in disease.

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Figure 1. Molecular mechanisms associated with activation of mI_{CAT} and smooth muscle contraction. Activation of M2 and M3 muscarinic receptors is coupled to signal transduction events via G_{i/o} and G_{q/11} pathways leading to activation of adenylyl cyclase (AC) and PLC. These events result in generation of intracellular messengers cyclic AMP (not indicated in figure) and DAG as well as [Ca²⁺]_i increase owing to IP₃-mediated Ca²⁺ release via the inositol trisphosphate receptor (IP₃R). TRPC4 and TRPC6 channels are activated as a consequence. TRPC6 is primarily activated by DAG, whereas the activation of TRPC4 depends on G_{i/o} and the channel is also subject to modulation by PIP₂ as well as [Ca²⁺]_i. The channels allow influx of Na⁺ and Ca²⁺, resulting in depolarization of the cells that in turn leads to activation of voltage-dependent calcium channels (VDCC), which provide the bulk of the Ca²⁺ required for contraction. Thus, these initial signaling events are the key determinants of intestinal motility.