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Molecular Expression and Functional Role of Canonical Transient Receptor Potential Channels in Airway Smooth Muscle Cells

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Abstract

Multiple canonical or classic transient receptor potential (TRPC) molecules are expressed in animal and human airway smooth muscle cells (SMCs). TRPC3, but not TRPC1, is a major molecular component of native non-selective cation channels (NSCCs) to contribute to the resting $[Ca^{2+}]_i$ and muscarinic increase in $[Ca^{2+}]_i$ in freshly isolated airway SMCs. TRPC3-encoded NSCCs are significantly increased in expression and activity in airway SMCs from ovalbumin-sensitized/challenged “asthmatic” mice, whereas TRPC1-encoded channel activity, but not its expression, is largely augmented. The upregulated TRPC3- and TRPC1-encoded NSCC activity both mediate “asthmatic” membrane depolarization in airway SMCs. Supportively, tumor necrosis factor- α (TNF α), an important asthma mediator, increases TRPC3 expression, and TRPC3 gene silencing inhibits TNF α -mediated augmentation of acetylcholine-evoked increase in $[Ca^{2+}]_i$ in passaged airway SMCs. In contrast, TRPC6 gene silencing has no effect on 1-oleoyl-2-acetyl-sn-glycerol (OAG)-evoked increase in $[Ca^{2+}]_i$ in primary isolated cells. These findings provide compelling information indicating that TRPC3-encoded NSCCs are important for physiological and pathological cellular responses in airway SMCs. However, continual studies are necessary to further determine whether, which, and how TRPC-encoded channels are involved in cellular responses in normal and diseased (e.g., asthmatic) airway SMCs.

38.1 Introduction

The canonical or classic transient receptor potential (TRPC) channels are encoded by genes that are most closely related to the *trp* gene that was originally identified in *Drosophila*. Photoreceptor cells in *Drosophila* produce a transient receptor potential, consisted of an initial rapid spike followed by a sustained phase in response to light. These two phases of a receptor potential are mediated by TRP-encoded and TRP-like channels. The first mammalian TRP gene was cloned from the human brain using an expressed sequence tag, categorized into the TRPC family based on primary amino acid sequence, and thus termed TRPC1. Currently, the TRPC family is known to consist of seven members designated TRPC1-7. In addition to the TRPC family, many other mammalian TRP molecules have been discovered and classified into the following five different subfamilies based on their activation stimuli and homology: TRPV (V for vanilloid), TRPM (from the tumor

suppressor melastatin), TRPP (P for polycystin), TRPML (ML for mucolipin), and TRPA (A for ankyrin).

TRPC molecules may encode nonselective cation channels (NSCCs), playing important roles in physiological and pathological cellular responses in various types of cells [1, 2]. NSCCs are present in airway smooth muscle cells (SMCs). The activity of these channels is significantly increased in response to agonists to initiate and maintain intracellular Ca^{2+} signaling, which may play an important role in various cellular responses including contraction, proliferation, migration, and gene expression in airway SMCs. We and other investigators have started to explore which TRPC molecules may encode NSCCs and show functional importance in airway SMCs. Studies have yielded interesting data, greatly improving our current knowledge in the field. In this chapter, we highlight the important findings of studies on TRPC-encoded NSCCs in airway SMCs and discuss the fundamental questions that remain to be addressed as well.

38.2 Multiple TRPC Molecules are Expressed in Airway SMCs

An earlier study using a standard *reverse transcription*-polymerase chain reaction method has shown that TRPC1, TRPC2, TRPC3, TRPC4, TRPC5 and TRPC6 mRNAs are expressed in primary isolated guinea-pig airway SMCs [3]. However, another study has reported that TRPC1, TRPC3, TRPC4 and TRPC6, but not TRPC2 and TRPC5 mRNAs are detected in passaged human airway SMCs [4]. It has also been found that TRPC1, TRPC3, TRPC4, TRPC5 and TRPC6, but not TRPC2 mRNAs are present in passaged human airway SMCs [5]. Using Western blot analysis or immunofluorescence staining, previous studies further reveal expression of TRPC1, TRPC3 and TRPC6 proteins in primary isolated guinea pig airway SM cells and tissues [3, 6], TRPC1, TRPC3 and TRPC4 proteins in freshly isolated porcine airway SM tissues [7], TRPC6 protein in primary isolated guinea pig and passaged human airway SMCs [4, 8], and TRPC1, TRPC3, TRPC4, TRPC5 and TRPC6 proteins in passaged human airway SMCs [5]. Our recent studies unveil that TRPC1 and TRPC3 mRNAs and proteins are expressed in freshly isolated and primary mouse airway SMCs [9]. As summarized in Table 38.1, mRNAs and proteins of TRPC1 and TRPC3, but not other members, are expressed in freshly isolated, primary isolated and passaged airway SMCs.

38.3 TRPC3 Is an Important Molecular Component of Native Constitutively-Active NSCCs in Airway SMCs

Using the cell-attached single channel recording, a number of researchers have found that native constitutively-active channels are present in freshly isolated bovine and human airway SMCs [10–13]. These channels are blocked by the substitution of extracellular Na^+ with the non-permeable cation N-methyl-D-glucamine (indicating that the channels are Na^+ -permeable), but unaffected by inhibition of Ca^{2+} -activated or voltage-activated K^+ channels with iberiotoxin, tetraethylammonium or 4-aminopyridine. Substitution of extracellular Na^+ with Ca^{2+} does not change the channel slope conductance, but rather shifts its reversal potential. Thus, the identified constitutively-active single channels in bovine and human AMSCs are the NSCCs, which are permeable to both Na^+ and Ca^{2+} , with a higher permeability to the former ion than the later.

We have further characterized NSCCs in freshly isolated mouse airway SMCs using the excised inside-out single channel recording [9]. These channels are constitutively active and can maintain their activity for up to 20 min without a time-dependent decrease. The relationship between the single channel current amplitudes and membrane potentials shows that the channels have 15, 37 and 57 pS conductance states. All three conductance states

have the same reverse potential of ~ 0 mV. Cell-attached single channel recordings have also observed the ~ 15 pS NSCCs in bovine and human airway SMCs [10–13]. The inability to detect the two other conductances may be due to the different modes of single channel recordings; as such, the activity of native constitutively-active NSCCs is more readily recorded using the excised, rather than cell-attached recording, mode. The channel activity in freshly isolated mouse airway SMCs is significantly higher at positive than negative potentials, suggesting that the native NSCCs exhibit outward rectification in airway SMCs. A diacylglycerol analogue, 1-oleoyl-2-acetyl-sn-glycerol (OAG), significantly increases the channel activity. The channel activity is augmented by elevating extracellular Ca^{2+} concentration and inhibited by reducing extracellular Ca^{2+} concentration. Thus, constitutively-active NSCCs in airway SMCs possess diacylglycerol- and Ca^{2+} -gated properties, which are very similar to native constitutively-active NSCCs in vascular SMCs [14, 15].

Our recent study also reveals that application of specific TRPC3 antibodies blocks the activity of native single NSCCs by $\sim 80\%$ in freshly isolated airway SMCs [9]. TRPC3 gene silencing by specific siRNAs inhibits the single channel activity to a very similar extent in primary isolated cells. These patch clamp and genetic studies for the first time provide compelling evidence to reveal that native constitutively-active NSCCs are mainly encoded by TRPC3 in airway SMCs. These findings, together with the fact that the three conductance states of the channels exhibit the same reversal potentials, also suggest that TRPC3 may form homomeric and/or heteromeric NSCCs that have the different conductance states. TRPC1 is not involved in the formation of these channels, as its antibodies and siRNAs have no effect on the channel activity. More recently, we have found that application of specific TRPC4 antibodies does not produce an inhibitory effect (Xiao and Wang, unpublished data). As shown in Table 38.2, each of the TRPC channels has different biophysical and pharmacological properties. The properties that individual channels manifest may be determined by intracellular regulatory molecules and experimental conditions. Thus, further work is necessary to determine the biophysical and pharmacological characteristics of TRPC3 and other potential TRPC channels in airway SMCs.

38.4 TRPC3-Encoded NSCCs are Important for Controlling the Resting Membrane Potential and $[\text{Ca}^{2+}]_i$ in Airway SMCs

The resting membrane potential (V_m) in airway SMCs is between -40 and -50 mV, similar to other types of SMCs, significantly less negative than a K^+ equilibrium potential of ~ -85 mV [16, 17]. Consistent with these previous reports, we have also found that the resting V_m is -44 mV in freshly isolated airway SMCs [9]. More importantly, our data reveal that specific TRPC3 antibodies and gene silencing both result in a pronounced V_m hyperpolarization by ~ 14 mV. Conversely, TRPC1 antibodies and gene silencing have no significant effect on the resting V_m . Thus, TRPC3-encoded NSCCs are important for controlling the resting V_m in airway SMCs, consistent with the central role of TRPC3 in mediating the activity of constitutively-active NSCCs. It is interesting to point out that constitutively-active NSCCs contribute to the less negative V_m than the K^+ equilibrium potential of ~ -85 mV in coronary and pulmonary artery SMCs [18, 19]. These data, together with the fact that TRPC3 is a major molecular constituent of native constitutively-active NSCCs in vascular SMCs [20], suggest that TRPC3-formed NSCCs are most likely to regulate the resting V_m as well in vascular SMCs. However, this view needs confirmation through further experimentation. In addition, whether other TRPC members are involved in the control of the less negative V_m in airway and vascular myocytes remain to be determined.

Comparable to the effect on the resting membrane potential, we have found that siRNA-mediated TRPC3 gene silencing significantly lowers the resting $[Ca^{2+}]_i$ in primary isolated airway SMCs [9]. However, TRPC1 gene silencing does not alter the resting $[Ca^{2+}]_i$. These findings provide evidence that TRPC3, but not TRPC1-encoded NSCCs, play an important role in the control of the resting $[Ca^{2+}]_i$ in airway SMCs.

38.5 TRPC3-Encoded NSCCs May Mediate Agonist-Induced Increase in $[Ca^{2+}]_i$ in Airway SMCs

Whole-cell patch clamp studies demonstrate that muscarinic agonists acetylcholine and methacholine activate NSCCs in freshly isolated canine, equine, guinea-pig and swine airway SMCs [21–25]. Simultaneous measurements of membrane currents and $[Ca^{2+}]_i$ reveal that activation of NSCCs during muscarinic stimulation is always accompanied by a sustained increase in $[Ca^{2+}]_i$ due to extracellular Ca^{2+} influx [22–25]. The sustained increase in $[Ca^{2+}]_i$ induced by methacholine and other agonists are largely inhibited or abolished by the general NSCC blockers Ni^{2+} , Cd^{2+} , La^{3+} , Gd^{3+} and SKF-96365 [12, 23, 25–32]. Thus, functional NSCCs mediate agonist-induced Ca^{2+} influx and associated increase in $[Ca^{2+}]_i$ in airway SMCs.

We and other investigators have started to identify which of the TRPC-encoded NSCCs are responsible for agonist-induced increase in $[Ca^{2+}]_i$. Our recent study reveals that TRPC3 gene silencing inhibits methacholine-evoked increase in $[Ca^{2+}]_i$ in primary isolated airway SMCs. Differently, TRPC1 gene silencing does not produce an effect. Similar to our findings, a previous report has shown that TRPC3 gene silencing blocks acetylcholine- and tumor necrosis factor- α (TNF- α)-induced increase in $[Ca^{2+}]_i$ as well in passaged human airway SMCs [5]. Moreover, OAG, which is known to be a putative activator for TRPC-encoded channels [1, 2], causes a significant increase in $[Ca^{2+}]_i$ in primary isolated guinea pig airway SMCs; however, TRPC6 gene silencing does not affect OAG-evoked response [8]. Collectively, TRPC3-encoded NSCCs are important for agonist-induced Ca^{2+} responses in airway myocytes. Relative to Ca^{2+} release from the sarcoplasmic reticulum (SR, a major intracellular Ca^{2+} store), extracellular Ca^{2+} influx through TRPC3-encoded NSCCs makes a smaller contribution to agonist-induced initial increase in $[Ca^{2+}]_i$. On the other hand, this TRPC3-mediated Ca^{2+} signaling is persistent during agonist stimulation. As such, this persistent Ca^{2+} signaling may be essential for maintaining cell contraction and other cellular responses, as well as refilling intracellular Ca^{2+} stores to start a new response.

Our previous studies have shown that the muscarinic activation of NSCCs in airway SMCs is mediated by a pertussis toxin-sensitive, Gi/o protein-coupled M_2 receptor signaling pathway [22]. It is noted that the same signaling pathway is involved in the muscarinic activation of expressed TRPC4 channels in HEK293 cells [33]. Furthermore, Flockerzi and his colleagues have recently reported that NSCCs in intestinal myocytes, which show similar biophysical properties and activation mechanisms to those in airway SMCs, are mainly mediated by TRPC4 [34]. Hence, it would be interesting to conduct further studies to determine the role of TRPC-encoded NSCCs in mediating agonist-induced increase in $[Ca^{2+}]_i$ in airway SMCs.

38.6 TRPC-Encoded NSCCs Cause Ca^{2+} Influx Through Themselves Directly, Na^+/Ca^{2+} Exchanger-1 and/or L-type Ca^{2+} Channels in Airway SMCs

A previous study using the whole-cell patch clamp recording with cation substitution has shown that extracellular Ca^{2+} can pass through NSCCs in freshly isolated airway SMCs during muscarinic stimulation [21]. In addition, our simultaneous measurements of membrane currents and $[Ca^{2+}]_i$ further reveal that under physiological conditions, a direct

Ca^{2+} permeability of NSCCs contributes to 14% of the associated muscarinic Ca^{2+} influx in freshly isolated airway SMCs [23]. Thus, functional NSCCs mediate agonist-induced increase in $[\text{Ca}^{2+}]_i$ in airway SMCs by directly permitting Ca^{2+} influx through the opening channels, as diagrammed in Fig. 38.1.

The plasmalemmal $\text{Na}^+/\text{Ca}^{2+}$ exchangers play an important role in the control of intracellular Ca^{2+} homeostasis in many types of cells. These exchangers can operate in the forward mode (also termed the Ca^{2+} removal mode) to remove one Ca^{2+} ion out of the cell in exchange for three Na^+ ions entering the cell, or in the reverse mode (Ca^{2+} entry mode) to bring one Ca^{2+} ion into the cell in exchange for three Na^+ ions leaving the cell. The ion transport direction and activity of $\text{Na}^+/\text{Ca}^{2+}$ exchangers depend on the electrochemical gradient of both Ca^{2+} and Na^+ ions as well as membrane potential [35, 36]. TRPC-encoded NSCCs exhibit a high permeability to Na^+ and may thus result in substantial Na^+ entry, which makes $\text{Na}^+/\text{Ca}^{2+}$ exchangers operate in the reverse mode, leading to Ca^{2+} influx (Fig. 38.1). In agreement with this view, an earlier study has found that reducing extracellular Na^+ from 137 to 5 mM or application of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger inhibitor KB-R9743 almost completely blocks TRPC3-mediated extracellular Ca^{2+} influx in HEK293 cells [37]. Extracellular Ca^{2+} influx is also inhibited by dominant negative suppression of TRPC3 channel function. Low density membrane fractionation and co-immunoprecipitation experiments reveal the co-localization of TRPC3 and $\text{Na}^+/\text{Ca}^{2+}$ exchanger-1, and glutathione *S*-transferase pull-down experiments demonstrate that $\text{Na}^+/\text{Ca}^{2+}$ exchanger-1 binds to TRPC3 C-terminus. Moreover, several reports have shown that the $\text{Na}^+/\text{Ca}^{2+}$ exchanger blocker KB-R7943 and the NSCC inhibitor SKF-96365 both largely inhibit or abolish agonist-evoked increase in $[\text{Ca}^{2+}]_i$ in freshly isolated airway SMCs and muscle contraction in freshly isolated airway strips [28–32, 38]. These results suggest that $\text{Na}^+/\text{Ca}^{2+}$ exchangers are important for the role of TRPC-encoded NSCCs in mediating Ca^{2+} influx and associated cellular responses in airway SMCs.

Significant Na^+ entry through TRPC-encoded NSCCs may also cause membrane depolarization and then activation of L-type Ca^{2+} channels, contributing to Ca^{2+} influx (Fig. 38.1). Supportively, the whole-cell patch clamp recording in combination with measurement of $[\text{Ca}^{2+}]_i$ has shown the presence of L-type Ca^{2+} channels and associated Ca^{2+} influx in freshly isolated bovine, canine, equine, guinea pig and human airway SMCs [39–46]. A number of studies have found that a sustained increase in $[\text{Ca}^{2+}]_i$ and associated contraction following application of muscarinic and other agonists are inhibited by the L-type Ca^{2+} channel blockers nifedipine and verapamil in bovine, canine and mouse airway SMCs [29, 30, 38, 47–51]. In contrast, it has been reported that agonist-induced contraction is not significantly affected by L-type Ca^{2+} channel blockers in airway SMCs [51–55]. It should also be noted that clinical studies indicate that L-type Ca^{2+} channel blockers are ineffective in the treatment of asthma [56–63]. Furthermore, our recent work demonstrates that membrane depolarization causes a direct activation of G protein-coupled receptors, leading to Ca^{2+} release and contraction without the involvement of L-type Ca^{2+} channel in airway SMCs [46]. Thus, the significance of L-type Ca^{2+} channels in the role of TRPC-encoded NSCCs in regulating agonist-induced Ca^{2+} influx needs to be further evaluated in airway SMCs.

38.7 TRPC-Encoded NSCC-Mediated Ca^{2+} Influx Occurs Due to Store-Operated Ca^{2+} Entry, Receptor-Operated Ca^{2+} Entry, or Both Processes in Airway SMCs

Agonist-induced, NSCC-mediated Ca^{2+} influx exhibits two distinct signaling processes: store-operated Ca^{2+} entry (also called capacitance Ca^{2+} entry) and receptor-operated Ca^{2+}

entry. Stimulation of muscarinic receptors or other G-protein coupled receptors result in activation of phospholipase C and generation of inositol 1,4,5-trisphosphate (IP₃). IP₃ binds to and activates its receptors, inducing Ca²⁺ release from the SR, which plays an important role in cellular responses. On the other hand, IP₃-mediated Ca²⁺ release leads to a decrease in the SR Ca²⁺ and subsequently store-operated Ca²⁺ entry. The NSCCs mediating this ubiquitous Ca²⁺ entry process are proposed to be essential for agonist-induced sustained increase in [Ca²⁺]_i and for the refilling of the SR Ca²⁺ store in airway SMCs [17, 64, 65]. It has been shown that after depletion of the SR Ca²⁺ store with thapsigargin or cyclopiazonic acid in the absence of extracellular Ca²⁺, readmission of extracellular Ca²⁺ results in store-operated Ca²⁺ entry in primary isolated and passaged airway SMCs [5, 13, 66–70]. Whole-cell patch clamp recordings indicate that cyclopiazonic acid generates store-operated NSCC currents [13, 66, 70, 71]. A number of studies have reported that store-operated Ca²⁺ entry is significantly increased by muscarinic and other agonists [5, 7, 66, 68]. Moreover, it has been found that incubation of TNF for hours significantly increases store-operated Ca²⁺ entry in passaged human airway SMCs, and the effect of TNF is attenuated by TRPC3 gene silencing [5]. These data indicate the direct activation of store-operated NSCCs and associated Ca²⁺ entry in airway SMCs during agonist stimulation, which can be mediated by TRPC3.

Activation of phospholipase C following stimulation of muscarinic receptors and other G-protein-coupled receptors can also produce diacylglycerol and its derivatives (e.g., arachidonic acid). These products lead to NSCC-mediated receptor-operated Ca²⁺ entry independent of depletion of the SR Ca²⁺ in many types of cells [1, 2]. Application of OAG, a diacylglycerol analogue, causes an increase in [Ca²⁺]_i in primary isolated guinea pig airway SMCs [8]. These results suggest that receptor-operated NSCCs and associated Ca²⁺ influx exist, contributing to agonist-induced increase in [Ca²⁺]_i and contraction in airway SMCs. In support, methacholine or histamine can cause a further increase in [Ca²⁺]_i and tension in freshly isolated guinea pig tracheal rings under the condition that store-operated Ca²⁺ entry is fully activated by thapsigargin [69]. We and others have discovered that TRPC3 gene silencing significantly reduces the muscarinic increase in [Ca²⁺]_i in primary isolated mouse and passaged humans airway SMCs [5, 9]. It has also been reported that TRPC6 gene silencing has no effect on OAG-induced increase in [Ca²⁺]_i in primary isolated guinea pig airway myocytes [8]. Collectively, TRPC3-, but not TRPC6-encoded, NSCCs are likely to be involved in receptor-operated Ca²⁺ entry following stimulation of G-protein-coupled receptors.

38.8 TRPC3-Encoded Native Constitutively-Active NSCCs are Significantly Upregulated in Expression and Activity in Asthmatic Airway SMCs

Asthma is a chronic lung disease characterized with two distinct features: airway hyperresponsiveness and inflammation. Both asthmatic airway hyperresponsiveness and inflammation are closely associated with an abnormal increase in Ca²⁺ signaling in airway SMCs. Simultaneous measurements of [Ca²⁺]_i and contraction have shown that the increased contraction following exposure to spasmodogens is nicely mirrored by an increased rise in [Ca²⁺]_i in freshly isolated asthmatic rabbit airway muscle strips [72]. The potentiation of spasmodogen-induced increase in [Ca²⁺]_i has also been found in freshly isolated hyperresponsive rat airway SMCs [73–75]. Moreover, the removal of extracellular Ca²⁺ and inhibition of an increase in [Ca²⁺]_i with various pharmacological agents attenuate or prevent asthmatic airway muscle hyperresponsiveness in animals [72, 76, 77]. There is compelling evidence that an abnormal alteration in [Ca²⁺]_i occurs in asthmatic human airway SMCs as well [5, 68, 78–83]. In addition, clinical studies demonstrate that drugs that interrupt intracellular Ca²⁺ mobilization can reverse airway muscle hyperresponsiveness [84–86]. Furthermore, the actions of glucocorticoids, α -adrenergic agonists and cholinergic blockers,

the most effective clinical drugs in the treatment of asthma, are related to their inhibition of abnormal increases in $[Ca^{2+}]_i$ in freshly isolated and passaged airway SMCs during asthmatic stimuli [64, 65, 87–90]. Collectively, it is reasonable to state that the enhanced increase in $[Ca^{2+}]_i$ in airway SMCs is an important converging pathway for asthmatic airway hyperresponsiveness and inflammation as well.

To explore the potentially significant contribution of TRPC-encoded NSCCs in the development of asthma, White et al. have shown that TRPC3 mRNA and protein expression are significantly increased in passaged human airway SMCs following treatment with TNF α , an important asthma mediator [5]. These authors have further found that TRPC3 gene silencing inhibits TNF α -induced Ca^{2+} influx and associated increase in $[Ca^{2+}]_i$, as well as TNF α -mediated augmentation of acetylcholine-evoked increase in $[Ca^{2+}]_i$. All these findings suggest that TRPC3-encoded NSCCs may be upregulated in the molecular expression and function role, contributing to the asthmatic cellular responses in airway SMCs. However, there is a lack of direct experimental evidence; thus, we have begun to determine the potential changes in TRPC molecule expression and functional activities in asthmatic airway SMCs. Using ovalbumin-sensitized/challenged “asthmatic” mice, we have shown that TRPC1 protein expression level is not changed in freshly isolated “asthmatic” airway SM tissues [9]. On the other hand, TRPC3 protein expression level is increased by threefolds.

Comparable to airway muscle hyperresponsiveness in vitro and in vivo, the activity of native NSCCs is increased by fourfolds in freshly isolated “asthmatic” airway SMCs [9]. The increased channel activity is completely blocked by specific TRPC1 antibodies. Similar to a previous report detailing “asthmatic” rat airway SMCs [16], our data unveil that membrane depolarization is found in freshly isolated “asthmatic” mouse airway SMCs. The “asthmatic” membrane depolarization is prevented by TRPC1 antibodies. In spite of having no clear explanation for these interesting but puzzling results, we would speculate that TRPC1-encoded NSCCs, which are dormant in normal cells, can be activated by an unknown mechanism in “asthmatic” cells. We have also revealed that the “asthmatic” increase in the activity of constitutively-active NSCCs and depolarization in membrane potential are both blocked by TRPC3 antibodies [9]. Thus, TRPC3-encoded native constitutively-active NSCCs are upregulated in both the molecular expression and functional activity, contributing to membrane depolarization and hyperresponsiveness in “asthmatic” airway SMCs, whereas TRPC1-encoded NSCCs are only functionally upregulated to mediate the increased cellular responses in “asthmatic” airway SMCs.

38.9 Perspective

We and other investigators have started to explore the molecular expression and potential functional roles of TRPC-encoded NSCCs in airway SMCs. A number of reports have shown that TRPC1-6 mRNAs and proteins are expressed in primary isolated and passaged airway SMCs [3–9]. Hitherto, there are only three studies to explore the functional importance of these channels in airway SMCs [5, 8, 9]. One of these three available functional studies has found that TNF α , an important asthma mediator, significantly increases TRPC3 mRNA and protein expression, and TRPC3 gene silencing inhibits TNF α -mediated augmentation of acetylcholine-evoked increase in $[Ca^{2+}]_i$ in passaged airway SMCs [5]. Another study indicates that TRPC6 gene silencing has no effect on OAG-evoked increase in $[Ca^{2+}]_i$ in primary isolated cells [8]. Finally, our recent patch clamp study, for the first time, provides evidence that TRPC3, but not TRPC1, is a major molecular component of native constitutively-active NSCCs in freshly isolated cells [9]. Further research using specific antibodies and gene silencing reveal that TRPC3-, but not TRPC1-encoded NSCCs show important functional roles in mediating the resting V_m and $[Ca^{2+}]_i$ as

well as muscarinic increase in $[Ca^{2+}]_i$ in primary isolated cells. We have also found that TRPC3 protein expression and encoded NSCC activity are both significantly increased in freshly isolated “asthmatic” airway SMCs. Interestingly, TRPC1 protein expression is unaltered, but its encoded channel activity is largely augmented. The upregulated TRPC3 and TRPC1-encoded NSCCs mediate membrane depolarization in “asthmatic” airway SMCs.

The findings described above provide novel and interesting information indicating that TRPC-encoded NSCCs may possibly contribute to asthmatic airway hyperresponsiveness and inflammation. However, due to limited studies, many fundamental questions remain unanswered. For instance, it is unknown whether other TRPC molecules, in addition to the identified TRPC3 [9], are also involved in the formation of constitutively-active NSCCs in airway SMCs. TRPC3 can be assembled with TRPC1 or TRPC6 to form heterotetrameric channels in vascular SMCs and other cell types [1, 2]. TRPC1 antibodies and gene silencing both have no effect on the activity of constitutively-active NSCCs in airway SMCs [9], indicating the lack of the involvement of this molecule. Regardless of this, future research is needed to investigate the possibility of TRPC6 or other TRPC members to form functional channels in airway SMCs.

TRPC-encoded NSCCs play an important role in the control of extracellular Ca^{2+} influx, contributing to the resting $[Ca^{2+}]_i$ and agonist-induced increase in $[Ca^{2+}]_i$ in airway SMCs [9]. Studies in other types of cells have shown that TRPC-encoded channels in mediating Ca^{2+} influx is determined by three distinct routes: the channels themselves (due to a direct Ca^{2+} permeability), Na^+/Ca^{2+} exchange-1 and L-type Ca^{2+} channels [1, 2]. However, there is no report to investigate which of these Ca^{2+} influx routes and to what extent they may participate in individual TRPC-encoded NSCC-mediated Ca^{2+} influx in airway SMCs. These important questions should be addressed in the near future. As TRPC-encoded NSCC-mediated Ca^{2+} influx occurs via two different signaling processes: store-operated and receptor-operated Ca^{2+} entry, it is also worthwhile to conduct a series of experiments to identify which of these processes is responsible for the role of individual TRPC-encoded NSCCs.

It should be pointed out that extensive attention has been paid to studies on Ca^{2+} signaling derived from TRPC-encoded NSCCs in many types of cells [1, 2], but not in airway SMCs. The low enthusiasm for studies on TRPC-encoded NSCCs in airway SMCs is possibly due to the following two main reasons. First, scientists in the field may question the functional importance of TRPC-encoded Ca^{2+} influx. However, current available information, even though it is limited, suggests that TRPC channels are potentially important for physiological and pathological cellular responses. Second, one may consider that much work has been done in other types of cells. The fact is that each type of organs and tissues are different in the structural, functional and molecular nature. For instance, the airway and vasculature – “the two preparations are quite different”; as such, “we should not look for short-cuts through the field of cardiovascular research” [91]. Thus, continual studies to determine whether, which, and how TRPC-encoded channels are involved in cellular responses in normal and diseased (e.g., asthmatic) airway SMCs are very necessary, which will yield novel and important findings to greatly enhance our understanding of the functional roles, regulatory mechanisms and signaling processes for TRPC-encoded NSCCs and also aid in the creation of effective therapeutic targets for the treatment of asthma and other respiratory diseases.

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Fig. 38.1.

A schematic diagram illustrates the three possible pathways for extracellular Ca^{2+} influx mediated by canonical transient receptor potential (TRPC)-encoded non-selective cation channels (NSCCs) in airway smooth muscle cells. First, TRPC-encoded NSCCs show a direct Ca^{2+} permeability and thus lead to Ca^{2+} influx through the opening channels. Second, TRPC channels are functionally coupled to $\text{Na}^+/\text{Ca}^{2+}$ exchanger-1 (NCX1), by which substantial Na^+ entry through TRPC-encoded NSCCs makes NCX1 operate in the reverse mode, causing Ca^{2+} influx to extrude Na^+ ions out of the cell. Finally, activation of TRPC-encoded NSCCs may result in membrane depolarization and then opening of L-type Ca^{2+} channels (LTCCs), leading to Ca^{2+} influx

Table 38.1

Profiles of molecular expression and functional roles of TRPC members in passaged, primary cultured (un-passaged) and freshly isolated airway SMCs

	TRPC1	TRPC2	TRPC3	TRPC4	TRPC5	TRPC6	TRPC7
Passaged	Guinea pig Human [3–6, 66]	Guinea pig (reference [3])	Guinea pig Human [3–5]	Guinea pig Human [3–5]	Guinea pig Human [3, 5]	Guinea pig Human [3–5]	N/A
Primary cultured	Mouse [9]	N/A	Mouse [9]	N/A	N/A	Guinea pig [8]	N/A
Freshly isolated	Mouse [9]	N/A	Mouse [9]	N/A	N/A	N/A	N/A

N/A, no data available.

Table 38.2

Biophysical and pharmacological properties of expressed TRPC channels in cell lines (modified from references [1, 20, 92])

	Conductance (pS)	Ion selectivity (PCa/PNa)	Constitutive activity	Activation	Inhibition	Binding partners
TRPC1	16, 24	1.0, Nonselective	–	PLC, OAG, stretch, store depletion	La^{3+} , Gd^{3+} , 2-APB, SKF-96365	TRPC3, TRPC4, TRPC5, IP_3R , CaM, Homer, Stim1
TRPC2	42	2.7 Nonselective	N/A	PLC, OAG, store depletion	La^{3+} , 2-APB, SKF-96365	IP_3R , CaM
TRPC3	42, 66	1.5, 1.6	+	PLC, OAG, Src kinase, IP_3 [Ca^{2+}] _i , store depletion	FFA, La^{3+} , Gd^{3+} , 2-APB, SKF-96365	TRPC6, TRPC7, IP_3R , CaM, RyR, NCX1
TRPC4	30, 42	1.1, 7.7	–	PLC, GTP S, receptor-operation, La^{3+} , store depletion,	La^{3+} , 2-APB, SKF-96365	TRPC1, TRPC5, IP_3R , CaM
TRPC5	38, 47, 66	1.8, 9.0	+	PLC, OAG, store depletion, Src kinase, IP_3 [Ca^{2+}] _i , PI3K, MLCK	FFA 2-APB, SKF-96365	TRPC1, TRPC4, CaM, MLCK
TRPC6	28, 35, 37	5.0	–	PLC, OAG, store depletion, Src, 20-HETE	FFA, La^{3+} , Gd^{3+} , 2-APB, SKF-96365	TRPC3, TRPC7, CaM, FKBP12, Fyn kinase
TRPC7	25, 50, 75	2	+	PLC, OAG, store depletion, 20-HETE	La^{3+} , Gd^{3+} , 2-APB, SKF-96365	TRPC3, TRPC6, CaM, FKBP12

2-APB, 2-aminoethoxydiphenyl borate; CaM, calmodulin; FKBP12, FFA, flufenamic acid, FK506 binding protein 12; 20-HETE, 20-hydroxyeicosatetraenoic acid; IP_3R , inositol 1,4,5-trisphosphate receptors; MLCK, myosin light chain kinase; NCX1, $\text{Na}^+/\text{Ca}^{2+}$ exchanger-1; OAG, 1-oleoyl-2-acetyl-sn-glycerol; PI3K, phosphoinositide-3-kinase; PLC, phospholipase C; RyR, ryanodine receptor.