

Review

# Calcium-Dependent Pulmonary Inflammation and Pharmacological Interventions and Mediators

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**Simple Summary:** Pulmonary diseases such as asthma, chronic obstructive pulmonary disease (COPD) and acute respiratory disease syndrome (ARDS) are common throughout the world. Tobacco products can potentially lead to lung damage, which in turn contribute to worsening disease outcomes. The same can be said of E-cigarette (E-cig) use, which has recently gained attention due to its possible adverse side effects. Despite this information, little is known about the potential for causality between smoking and E-cig use, and lung damage and worsening disease outcomes. In this review, we focus on a potential inflammatory mechanism, that is, calcium (Ca<sup>2+</sup>) signaling and its importance in many normal biological processes as well as roles it may play in the adverse effects of tobacco and E-cig use. In addition, we discuss possible therapeutic small molecules that may improve pulmonary disease outcomes in the future.



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**Abstract:** Pulmonary diseases present a significant burden worldwide and lead to severe morbidity and mortality. Lung inflammation caused by interactions with either viruses, bacteria or fungi is a prominent characteristic of many pulmonary diseases. Tobacco smoke and E-cig use (“vaping”) are considered major risk factors in the development of pulmonary disease as well as worsening disease prognosis. However, at present, relatively little is known about the mechanistic actions by which smoking and vaping may worsen the disease. One theory suggests that long-term vaping leads to Ca<sup>2+</sup> signaling dysregulation. Ca<sup>2+</sup> is an important secondary messenger in signal transduction. Cellular Ca<sup>2+</sup> concentrations are mediated by a complex series of pumps, channels, transporters and exchangers that are responsible for triggering various intracellular processes such as cell death, proliferation and secretion. In this review, we provide a detailed understating of the complex series of components that mediate Ca<sup>2+</sup> signaling and how their dysfunction may result in pulmonary disease. Furthermore, we summarize the recent literature investigating the negative effects of smoking and vaping on pulmonary disease, cell toxicity and Ca<sup>2+</sup> signaling. Finally, we summarize Ca<sup>2+</sup>-mediated pharmacological interventions that could potentially lead to novel treatments for pulmonary diseases.

**Keywords:** calcium (Ca<sup>2+</sup>); inflammation; pulmonary biology; macrophages; smoking; vaping; pathology



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## 1. Introduction

Respiratory diseases such as pneumonia, acute respiratory disease syndrome (ARDS) [1] and chronic obstructive pulmonary disease (COPD) [2] carry a significant worldwide burden of morbidity and mortality. Pulmonary diseases are characterized by inflammation within one or both lungs and are typically caused by infection with viruses, bacteria or fungal pathogens [3,4]. The stages of pulmonary inflammation are typically initiated through the interaction of the microbe/microbial product with the airway epithelium. This interaction leads to a pro-inflammatory response mediated by

cytokines and chemokines as well as macrophages and activated neutrophils at the site of insult [5,6]. In most cases, a healthy immune response is able to clear the infection, and the inflammation resolves. However, certain pathologic conditions result in robust neutrophil activation, leading to pneumonia and other forms of acute lung injury (ALI) such as ARDS.

Tobacco smoking is one of the major risk factors for the development of pulmonary disease and dysfunction [2]. In the US alone, tobacco use has accounted for ~20 million premature deaths since the publication of the Surgeon General's report in 1964. Tobacco exposure triggers a number of inflammatory responses in the airways, which can manifest as a number of very diverse ailments and include more commonly thought of pulmonary ailments: airway inflammation, ALI, ARDS, COPD and lung cancer as well as inflammatory bowel diseases, which often present along with pulmonary diseases such as COPD [2,7,8]. At the same time, tobacco smoke and the use of electronic cigarettes (or "vaping") itself renders the smokers' and vapers' lungs more susceptible to microbial infection/burden and, therefore, to pulmonary disease [9,10]. Because smokers' airways are characterized by dehydrated mucus, many well-documented studies have investigated cystic fibrosis transmembrane conductance regulator-mediated regulation [11] and the critical role that calcium ( $\text{Ca}^{2+}$ ) signaling and, in particular, abnormal  $\text{Ca}^{2+}$  influx plays in cystic fibrosis [12–15]. Both acute and chronic exposure to cigarette smoke lead to a chronic elevation in intracellular  $\text{Ca}^{2+}$  levels, and smoke from similar tobacco products is expected to exert similar effects, resulting in pulmonary disease initiation and progression [12,13,16]. Although  $\text{Ca}^{2+}$  dysregulation was shown to play a pivotal role in inflammation and to result in acute pulmonary disease, little is known about the mechanisms of  $\text{Ca}^{2+}$ -induced inflammatory lung diseases within the context of epithelial and endothelial cell function. Furthermore, abnormal  $\text{Ca}^{2+}$  homeostasis has itself been linked to the same pathologies as tobacco smoke, i.e., inflammation, ALI, ARDS, COPD and lung cancer [17].

As can be inferred from above, tobacco smoke exerts multiple effects on the airway epithelia and elevates intracellular  $\text{Ca}^{2+}$  ( $[\text{Ca}^{2+}]_i$ ), leading to dysfunctional ion secretion. When agonists bind to ion channels, the channels open to allow the flow of cation ions ( $\text{Na}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{K}^+$ ) through the cell membrane, inducing a wide variety of biological responses. Given the large number of physiological processes governed by changes in  $[\text{Ca}^{2+}]_i$ ,  $\text{Ca}^{2+}$  signaling clearly plays a pivotal role in pulmonary disease [12]. In fact, a sustained elevation of the basal  $\text{Ca}^{2+}$  concentration likely leads to cell stress, activating autophagy and cell death [18,19]. Herein, we will provide a detailed understanding of (1) the role of  $\text{Ca}^{2+}$  and  $\text{Ca}^{2+}$  signaling under basal physiological conditions, and (2)  $\text{Ca}^{2+}$  dysregulation as a mechanism of pulmonary disease development and progression with specific emphasis on tobacco products, which we focus upon due to their status as the major risk factor for pulmonary disease development.

## 2. $\text{Ca}^{2+}$ and Signal Transduction

$\text{Ca}^{2+}$  is an important secondary messenger within cells, triggering many cellular processes. Various cellular compartments utilize and manipulate  $\text{Ca}^{2+}$  concentrations. In terms of complexity, there exists a series of pumps, channels, transporters and exchangers that mediate intracellular and extracellular  $\text{Ca}^{2+}$  concentrations [20]. This complex system allows for the manipulation of  $\text{Ca}^{2+}$  to trigger numerous intracellular processes including cell metabolism, proliferation, death, gene transcription, secretion, etc. [20,21]. For example,  $\text{Ca}^{2+}$  mobilization generates signals based on its release from endoplasmic reticulum (ER) stores and the influx of extracellular  $\text{Ca}^{2+}$  [22].  $\text{Ca}^{2+}$  signaling utilizes multiple channels to mediate the cellular uptake of extracellular  $\text{Ca}^{2+}$  into cells. Among the most important of these channels are the Transient receptor protein channels (TRP), Store Operated Calcium Entry (SOCE) channels and Voltage-Gated Calcium (VGC) channels [20]. Thus, the activation of these channels is a major factor in regulating the uptake of  $\text{Ca}^{2+}$  into the cytosol of non-neuronal cells.

### 2.1. Transient Receptor Potential Channels

Transient receptor potential channels (TRP) are encoded in humans via six different orthologous genes (TRPC1-7 with the exclusion of TRPC6) [23]. TRP channels can be activated by depletion of internal stores of  $\text{Ca}^{2+}$  and exhibit significant diversity in terms of specificity and mechanisms of activation compared with other ion channels. These channels allow cells to respond to external stimuli as well as to sense changes in the local environment. There are many subfamilies of TRP channels that are activated and regulated in different ways. However, all TRPs share common activation cues through pathways that couple phospholipase C (PLC). Humans express six of the seven TRPC proteins due to *TRPC2* being a pseudogene [24]. All human TRPs use PLC for activation, though there is variability in specificity for  $\text{Ca}^{2+}$  and other cations. For example, many TRPs are activated upon the depletion of internal  $\text{Ca}^{2+}$  stores through  $\text{IP}_3$  or thapsigargin [23]. Interactions between ER  $\text{Ca}^{2+}$  release channels and TRPs have previously been described. However, TRP channels may still be active even after ER-resident  $\text{Ca}^{2+}$  channels, i.e.,  $\text{IP}_3\text{R}$  homologs, are knocked out, which suggests another mechanism of activation [8]. Thus, the current literature hypothesizes a complex relationship between  $\text{Ca}^{2+}$  store depletion and TRP activity and that a conformational coupling mechanism exists between TRPCs and ER-resident  $\text{Ca}^{2+}$  release channels. This mechanism would require a close but separate interaction between the ER and plasma membranes. Pharmacological reorganization of the actin cytoskeleton was also shown to decrease channel activity either by internalization or through the inactivation of exogenously expressed TRPC3 channels [23,25,26].

### 2.2. Store-Operated Calcium Entry

Store-operated  $\text{Ca}^{2+}$  entry (SOCE) is activated by the depletion of  $\text{Ca}^{2+}$  from ER-resident stores. In addition to refilling ER  $\text{Ca}^{2+}$  stores, SOCE channels also increase cytosolic  $\text{Ca}^{2+}$  levels to serve wider signaling functions [27,28]. The most prominent configuration for this channel is known as the  $\text{Ca}^{2+}$  release-activated  $\text{Ca}^{2+}$  (CRAC) current, which consists of the ER  $\text{Ca}^{2+}$  sensor STIM (Stromal Interaction Molecule) protein, and ORAI proteins, which are plasma membrane (PM) channels that are highly selective for increasing intracellular  $[\text{Ca}^{2+}]$  [29]. STIM proteins sense  $\text{Ca}^{2+}$  in the ER lumen and communicate  $\text{Ca}^{2+}$  store depletion to other proteins such as ORAI [6]. STIM causes the channel pore within ORAI to open, allowing  $\text{Ca}^{2+}$  to flow through the plasma membrane and into the cytoplasm. STIM1 and STIM2 proteins function by way of a low-affinity EF-hand  $\text{Ca}^{2+}$  binding site. STIM1 forms discrete PM-associated junctions and physically associates with ORAI1 and other ORAI channels, which are selective channels located within the plasma membrane [30,31]. STIM1 and STIM2 homologs are type-I membrane proteins with a luminal helix-turn-helix motif EF-hand  $\text{Ca}^{2+}$ -binding site as well as a sterile alpha motif (SAM) [31]. STIM1 localizes in the tubular ER but was detected in the plasma membrane where it is N-glycosylated. Evidence suggests that this N-glycosylation plays a role in plasma membrane delivery. Upon depletion of  $\text{Ca}^{2+}$  stores, STIM1 begins clustering and localizes to plasma membrane-adjacent ER regions [32]. This decrease in  $\text{Ca}^{2+}$  binding to the EF-hand specifically results in the clustering of STIM1 proteins followed by these multimers translocating to PM-adjacent ER areas where they can activate  $\text{Ca}^{2+}$  influx. ORAI proteins play a vital role in the CRAC current configuration. Three ORAI proteins also have varying functions in this pathway, which is despite their sequence similarity [31]. ORAI1 is thought to be most important in terms of  $\text{Ca}^{2+}$  influx into cells. Furthermore, decreasing its expression level has the most significant impact on SOCE [33,34]. In addition, ORAI1 and ORAI2 produce highly selective  $\text{Ca}^{2+}$  CRAC currents while ORAI3 produces small and developing CRAC currents [31,35]. The active pore of the channel consists of four ORAI molecules, which are induced by STIM1 interaction [31]. ORAI1 alone inhibits SOCE. Thus, the co-expression of STIM1 and ORAI1 is necessary to induce the influx of  $\text{Ca}^{2+}$  and the creation of CRAC channels [33,36].

### 2.3. Voltage-Gated $\text{Ca}^{2+}$ Channels

Voltage-gated  $\text{Ca}^{2+}$  channels (VGCC) are activated upon the depolarization of the membrane and mediate  $\text{Ca}^{2+}$  entry into the cell. This event generates electrical signals that mediate many cellular events, e.g., enzyme activation and gene expression [37]. VGCCs are key signal transducers that convert the action potential signal in the membrane to a transient intracellular  $\text{Ca}^{2+}$  signal. These  $\text{Ca}^{2+}$  currents vary by cell type as defined by pharmacological criteria [15]. L-type  $\text{Ca}^{2+}$  currents exhibit slow voltage-dependent inactivation and, as a result, are long-lasting when (1)  $\text{Ba}^{2+}$  is the carrier and (2) there is no  $\text{Ca}^{2+}$ -dependent inactivation. T-type  $\text{Ca}^{2+}$  channels are activated at more negative membrane potentials than L-type and are inactivated rapidly and deactivated slowly [38]. N-type  $\text{Ca}^{2+}$  channels have different intermediate voltage dependence and rate of inactivation when compared to L and N-type channels, which are more negative and faster than the L-type and more positive and slower than the T-type. Finally, there are L- and T-type  $\text{Ca}^{2+}$  currents, which are found in a variety of cells.

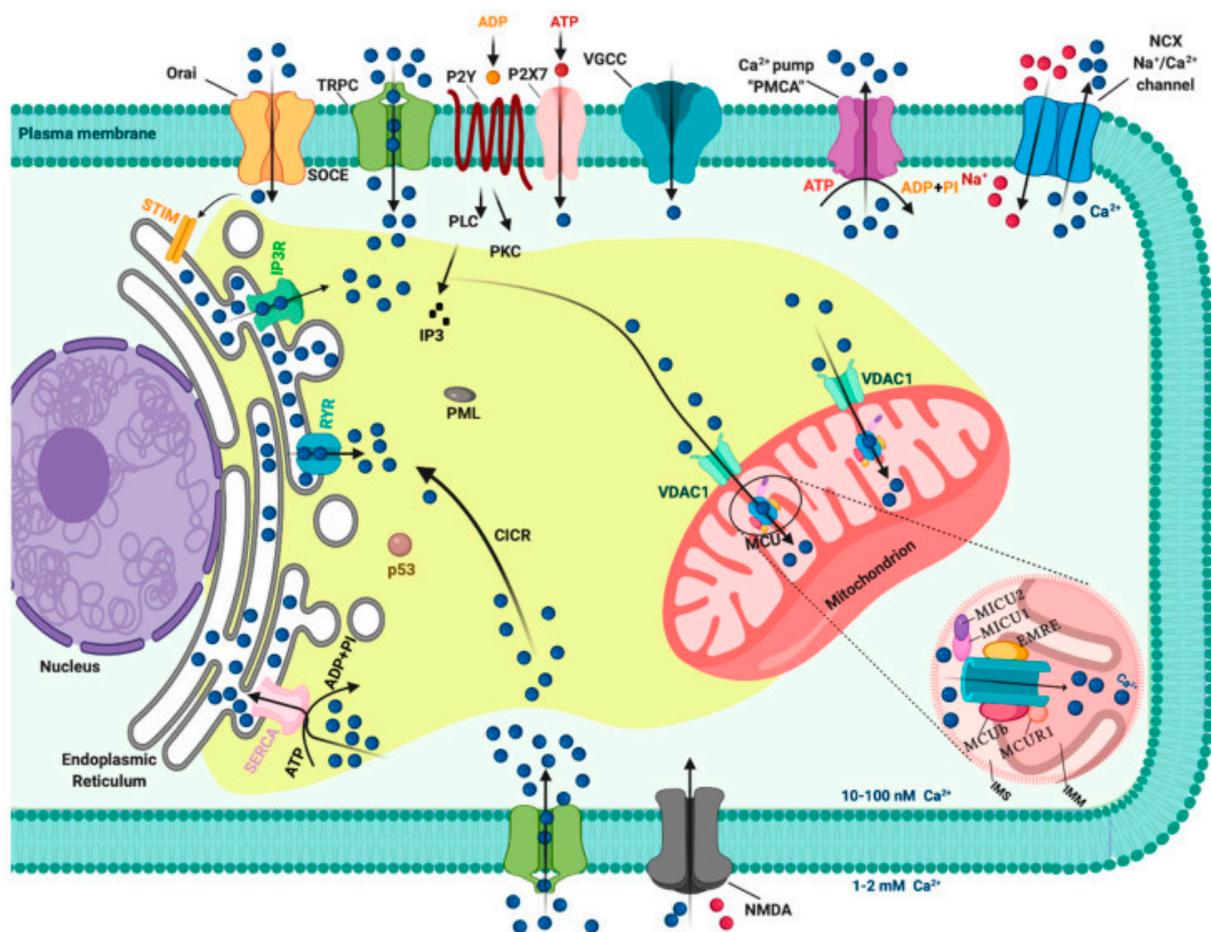
### 3. Additional $\text{Ca}^{2+}$ Signaling Components

Additional proteins serve as integral channel components for  $\text{Ca}^{2+}$  release and signaling:  $\text{Ca}^{2+}$  release through Inositol 1,4,5 triphosphate receptors ( $\text{IP}_3\text{R}$ ), which are located on the ER, can also drive increases in intracellular  $[\text{Ca}^{2+}]$  [20,39].  $\text{IP}_3\text{Rs}$  are activated when cell surface receptors are activated [40]. Following activation,  $\text{Ca}^{2+}$  is released from the ER, Golgi apparatus and nuclear envelope, and  $\text{IP}_3\text{Rs}$  localized to the plasma membrane are activated [41,42].  $\text{IP}_3$  production is triggered by external stimuli that bind to cell surface receptors. These binding events induce  $\text{Ca}^{2+}$  signaling within cells over a short period of time [21,43]. The pathway for  $\text{IP}_3$  production and  $\text{IP}_3\text{R}$  activation is referred to as the phosphoinositide signaling pathway. Phospholipase C (PLC), which interacts with many other  $\text{Ca}^{2+}$  signaling pathways, produces  $\text{IP}_3$  in cells and is expressed in different isoforms; however, only a few isoforms activate in response to external stimuli. Other isoforms may be activated by  $\text{Ca}^{2+}$ , G protein Ras or are introduced during fertilization [21]. The hydrolysis of  $\text{PIP}_2$  by PLC not only produces  $\text{IP}_3$  but also diacylglycerol (DAG), which is maintained within the plasma membrane and plays a role in other  $\text{Ca}^{2+}$  channels as well as in the activation of protein kinase C. Conversely, the DAG may be metabolized to produce other cellular messengers.

Ryanodine receptors (RyRs) share 40% homology with  $\text{IP}_3\text{Rs}$  and are  $\text{Ca}^{2+}$  release channels located on the sarcoplasmic reticulum and endoplasmic reticula [44]. In tissues, the influx of  $\text{Ca}^{2+}$  through plasma membrane  $\text{Ca}^{2+}$  channels activates the RyRs, resulting in rapid, massive releases of intracellular  $\text{Ca}^{2+}$  stores.

Sarcoendoplasmic reticulum  $\text{Ca}^{2+}$ -ATPase (SERCA) is responsible for pumping  $\text{Ca}^{2+}$  into the ER lumen. These receptors mediate the storage of  $\text{Ca}^{2+}$  in the ER. Voltage-dependent anion channels (VDAC) and the mitochondrial  $\text{Ca}^{2+}$  uniporter family mediate mitochondrial  $\text{Ca}^{2+}$  entry [20]. Figure 1 [20] illustrates the diversity of pathways mediated by intracellular  $\text{Ca}^{2+}$  signaling.

$\text{Ca}^{2+}$  signaling is not only important in intracellular processes but also plays a large role in immune cell response and inflammation.  $\text{Ca}^{2+}$  plays a pivotal role in cells as a secondary messenger. Stimulation of  $\text{Ca}^{2+}$  is responsible for the activation and regulation of many immune cells. Macrophages are key immune cells that function through phagocytosis. Their phagocytic function is dependent on internal  $\text{Ca}^{2+}$  regulation [45]. Increases in cytosolic  $\text{Ca}^{2+}$  are important for phagocytosis as well as phagosome maturation. In addition,  $\text{Ca}^{2+}$  binding to phagocytic receptors on macrophages triggers  $\text{IP}_3$  production. This release of  $\text{Ca}^{2+}$  from the ER triggers SOCE. The influx of extracellular  $\text{Ca}^{2+}$  is also required for macrophage polarization into the M1 state, which is pro-inflammatory and important for fighting pulmonary infections. Lowering the cytosolic  $\text{Ca}^{2+}$  concentration can modulate the switch of M1 macrophages to M2, which are termed anti-inflammatory.



**Figure 1.** Intracellular calcium ( $\text{Ca}^{2+}$ ) signaling. Different  $\text{Ca}^{2+}$  transporters, channels, exchangers, binding/buffering proteins and pumps mediate the regulation of the cytosolic  $\text{Ca}^{2+}$  concentration. In the plasma membrane (PM), PM  $\text{Ca}^{2+}$ -ATPases (PMCA) pumps, transient receptor potential channels (TRPC), voltage-gated  $\text{Ca}^{2+}$  channels (VGCC),  $\text{Na}^{+}/\text{Ca}^{2+}$  exchanger (NCX) and purinergic P2 receptors regulate the transport of  $\text{Ca}^{2+}$  ions inside and outside cells. Inositol 1,4,5-triphosphate receptors ( $\text{IP}_3\text{R}$ ), ryanodine receptors (RyR) and sarcoendoplasmic reticulum  $\text{Ca}^{2+}$ -ATPase (SERCA) pumps control the storage of  $\text{Ca}^{2+}$  in the endoplasmic reticulum. Finally, voltage-dependent anion channels (VDAC) and members of the mitochondrial  $\text{Ca}^{2+}$  uniporter family are critical for controlling the mitochondrial  $\text{Ca}^{2+}$  uptake. Abbreviations: CICR: Calcium-induced calcium release; PML: Promyelocytic leukemia protein; MICU1: Mitochondrial calcium uniporter component 1; MICU2: Mitochondrial calcium uniporter component 2; EMRE: Essential MCU regulator; MCUB: Calcium uniporter regulatory subunit; IMS: Intermembrane space; IMM: inner mitochondrial membrane. From [20] From Patergnani et al., 2020, with the original work cited as an open access article, thus allowing for use under the terms of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/> accessed on 26 July 2021).

In neutrophils,  $\text{Ca}^{2+}$  signaling is regulated mainly by SOCE channels. The influx of  $\text{Ca}^{2+}$  into the cytosol is responsible for neutrophil activation. In neutrophils, SOCE is initiated by ligand-receptor interactions, which trigger a complex cascade of molecules and results in ORAI1 stimulation. Stimulation of ORAI1 via STIM1 leads to  $\text{Ca}^{2+}$  influx, which increases the cytosolic  $\text{Ca}^{2+}$  concentration [46]. This results in the pro-inflammatory activation of neutrophils.

In lymphocytes,  $\text{Ca}^{2+}$  is present at low concentrations prior to activation [47]. Cross-linking of antigen receptors leads to the activation of phospholipase C, which breaks down phosphatidylinositol-4-5-bisphosphate to create inositol-1,4,5-trisphosphate ( $\text{IP}_3$ ) and diacylglycerol.  $\text{IP}_3$  binds to receptors, leading to the release of  $\text{Ca}^{2+}$  into the cytosol. This depletion of the internal  $\text{Ca}^{2+}$  store activates SOCE channels, which are lymphocytes' main mode of  $\text{Ca}^{2+}$  influx.  $\text{Ca}^{2+}$  release-activated channels (CRAC) are the most common channel configuration for SOCE found in lymphocytes.

Ca<sup>2+</sup> dysregulation is an attributable trait that was described for various diseases including pulmonary diseases such as asthma and ALI [48]. However, little is known about the mechanistic action of Ca<sup>2+</sup> dysregulation in these diseases.

#### 4. Smoking and Ca<sup>2+</sup> Derangement and Disease

COPD is an inflammatory lung disease, a major global health threat and is characterized by obstructed airflow within the lungs [49]. This obstruction of airways is progressive and often a result of toxic chemical exposure, leading to irregular inflammatory responses within the lungs [50]. This airway obstruction is not fully reversible and is caused by the progressive narrowing, destruction and removal of terminal bronchioles, which eventually results in emphysema [51]. ARDS is the result of a complex cascading process that develops as a result of ALI [52] and is characterized by acute respiratory failure because of increases in inflammation and pulmonary edema [5]. Many patients with ALI also exhibit cases of diffuse alveolar damage, which is an injury to the gas exchange surfaces within the lung and eventually results in damage to the blood–air barrier [53]. Asthma is an inflammatory lung disease characterized by pulmonary inflammation and airway obstruction, with the risk of developing asthma being higher among those who smoke and have smoked than those who have never smoked [54]. These various pulmonary diseases share the commonality that smoking is a major contributor to their resultant dysfunctional contraction profiles. Smoking and the use of other tobacco products present significant health risks and lead to these pulmonary diseases. Smoking exposes humans to numerous toxic chemicals, leading to worsened mobility and significant mortality due to the development of pulmonary diseases, as previously described. These activities result in inflammatory reactions within the airway, as well as alterations to innate immunity in the oral, nasal and airway passageways, which is in addition to changes in adaptive immunity [55].

Recently Ca<sup>2+</sup> signaling dysfunction has gained attention as a potential cause for increased morbidity and disease mortality, with cytosolic Ca<sup>2+</sup> concentrations increasing following smoking or the use of other tobacco products [12]. The study cited here demonstrated that Ca<sup>2+</sup> influx, as well as endoplasmic reticulum and mitochondrial stores, do not play a role in the Ca<sup>2+</sup> release caused by tobacco use. The study suggests that the Ca<sup>2+</sup> originated from lysosomes and was maintained in the cytosol during chronic tobacco use. Total particulate matter (TPM) from tobacco products induced an increase in Ca<sup>2+</sup> unrelated to extracellular Ca<sup>2+</sup> and did not require the activation of the IP<sub>3</sub> and/or TRP channel pathways. Another study [22] demonstrated that Ca<sup>2+</sup> influx in bronchial epithelial cells was decreased in smokers' lungs, which occurred simultaneously with a decrease in Ca<sup>2+</sup> release from the ER because of the differential expression of ORAI3. This differential expression inhibited SOCE channels. As alluded to earlier, these data suggest that ORAI3 expression might play a large role in Ca<sup>2+</sup> signaling impairment in smokers as well as in patients with COPD.

#### 5. Emerging Tobacco Products and Ca<sup>2+</sup> Derangement and Disease

Electronic cigarette (E-cig) use or “vaping” has become more prevalent in countries worldwide as it was initially portrayed as a safer alternative to cigarette smoking. Despite this information, little is known if long-term vaping will have similar effects to cigarette smoking. Recent studies have indicated possible adverse side effects associated with vaping [56,57]. For example, vaping is associated with increases in ALI and other pulmonary toxicities [58]. Vaping may expose humans to oxidant or reactive oxygen species (OX/ROS), which is suggested by the oxidant reactivity of E-cig aerosols [59]. OX/ROS are present in cigarette smoke and generate tars that lead to lung inflammation, which is the cause of many pulmonary diseases such as COPD, ALI and lung cancer [59]. The flavor chemicals contained within vaped e-liquid products may also play a determining role in any vaping-associated toxicity. Recent studies have utilized *in vitro* and *in vivo* models to better understand and study any potential interactions that these vaped aerosols may have

within the lung and any resultant pulmonary toxicity. In vitro cell viability studies utilizing various lung cells have shown marked decreases in cell viability. Recent examples include studies using A549 (Human lung epithelial cells) and Calu3 (non-small-cell lung cancer), which are ideal models for studying the airway epithelium. These studies noted significant cytotoxic effects and significant decreases in cell viability when exposing these cells to different e-liquids possessing various flavors [60,61]. In particular, “Mint” flavored JUUL e-liquid decreased cell viability, led to elevated levels of the pro-inflammatory cytokine IL-6 and increased levels of intracellular  $\text{Ca}^{2+}$  and Annexin V, with Annexin V being a well-described marker for apoptosis [61]. Other examples include a combined effect with cinnamon flavorings and nicotine, with the combination leading to a significantly greater decrease in cell viability than cinnamon alone in A549 cells, suggesting that nicotine and flavor combinations may exhibit synergistic activities [60]. Cinnamon-flavored products also produce slight increases of the pro-inflammatory cytokine IL-8 and anti-inflammatory cytokine MCP-1. Bengalli and colleagues [60] utilized a co-culture of the human lung epithelial cell line NCI-H441 and human pulmonary microvascular endothelial cell line HPMEC-ST1.6R to mimic the alveolar–blood barrier. During smoking or vaping, these lung epithelial cells were exposed to aerosols, which altered the integrity of the barrier and resulted in ALI and diffuse alveolar damage. The results from this study revealed that vaping flavored e-liquid products, specifically cinnamon- and menthol-containing, significantly decreased the integrity of the barrier. Recent research studies have also begun to interrogate the  $\text{Ca}^{2+}$  dysregulation caused by e-liquids/vaping based upon observations of similar health impacts to those resulting from the use of tobacco products. With  $\text{Ca}^{2+}$  being integral for many physiological responses and processes, it stands to reason that its dysregulation would have negative effects. Rowell and colleagues [62] screened several e-liquid flavors for induced changes in  $\text{Ca}^{2+}$  homeostasis using human bronchial epithelial cells. Banana pudding flavored e-liquid caused ER  $\text{Ca}^{2+}$  release and activated SOCE. In particular, long exposures to the e-liquid depleted ER  $\text{Ca}^{2+}$  stores and inhibited store-operated  $\text{Ca}^{2+}$  entry, suggesting that e-liquids may alter  $\text{Ca}^{2+}$  homeostasis through short- and long-term mechanisms. Vaping was also shown to have severe effects on disease and disease severity. For example, our lab [63] utilized a murine model to test the effects of vaping on the severity of the coronavirus-dependent pulmonary disease. By exposing mice to vaped e-liquids followed by infection with Mouse Hepatitis Virus (MHV), a murine tropic virus that produces SARs-like pneumonia in mice, preliminary data indicated an increase in the mortality and pathology of MHV-induced pulmonary infection. This study also suggests a complex role for  $\text{Ca}^{2+}$  mobilization in inflammation and respiratory disease development in relation to vaping. Additional studies [63] also indicate ALI when mice are exposed to vaped e-liquid. Co-exposure models of vaping and MHV as well as vaping and *Klebsiella pneumoniae* also indicate an increase in ALI, i.e., increased lung wet/dry ratio. These increases in wet/dry ratio were significant in co-exposure models. One theory surrounding vaping is that it may trigger M0 macrophage polarization to pro-inflammatory M1 macrophages [64]. These M1 macrophages would then lead to increased lung inflammation as well as play a prominent role in disease progression. Interestingly, this polarization process is also mediated by increases in intracellular  $\text{Ca}^{2+}$ . These data indicate the potential side effects of vaping on health and disease outcomes and underlie the need for awareness of these effects as vaping continues to rise in popularity amongst minors and young adults.

## 6. Pulmonary Disease and Pharmacological Interventions Using $\text{Ca}^{2+}$ Signaling Mediators

Recent research suggests  $\text{Ca}^{2+}$  signaling dysregulation is not only the mechanism of the chronic side effects due to smoking and vaping but also a driving factor in pulmonary disease. These data suggest that targets affected by inhibitors of certain  $\text{Ca}^{2+}$  channels may serve as novel therapeutic targets for mediating many pulmonary diseases.  $\text{Ca}^{2+}$  signaling can be inhibited by  $\text{Ca}^{2+}$  antagonists such as dihydropyridines, phenylalkylamines and

benzothiazepines [37]. Pyr6, Pyr10 and 2-APB are other known  $\text{Ca}^{2+}$  channel antagonists whose effects on  $\text{Ca}^{2+}$  signaling have been well-studied [65,66]. Pyr6, Pyr10 and 2-APB inhibit store-operated  $\text{Ca}^{2+}$  entry, non-store operated calcium signaling and  $\text{IP}_3\text{R}$ , respectively. The antagonist 2-APB has been documented to inhibit  $\text{IP}_3\text{R}$ -induced  $\text{Ca}^{2+}$  release [65]. Pyr6 and Pyr10 display marked  $\text{Ca}^{2+}$  inhibition in TRPC- and ORAI-mediated  $\text{Ca}^{2+}$  entry, respectively [66]. In the previously mentioned murine model [63], the effects of 2-APB on vaping and dual vaping and MHV exposure were investigated. Mice that received the 2-APB treatment displayed improved respiratory characteristics and function vs the mice only infected with MHV. These  $\text{Ca}^{2+}$  channel antagonists were also able to significantly decrease inflammatory cytokine production as well as decrease pulmonary inflammation in vape-treated mice receiving either 2-APB or Pyr10. This study suggests that the  $\text{Ca}^{2+}$  channel antagonists 2-APB, Pyr10 and Pyr6 have potential use as novel therapeutics for pulmonary disease. Table 1 (modified from [67]) lists the various compounds targeting  $\text{Ca}^{2+}$  signaling components and their mechanism of action. Many other compounds exist but have not yet been tested in relation to pulmonary disease, thus offering a potential future avenue of research focused on investigating these  $\text{Ca}^{2+}$  signaling mediators.

**Table 1.** Compounds targeting  $\text{Ca}^{2+}$  signaling components.

$\text{Ca}^{2+}$ Signaling Components	Pharmacological Compounds	Mechanistic Action
Transient Receptor Potential Channels	Pyr6	Inhibitor
	20-GPPD	Activator
	SKF96365	Blocker
	D-3263	Agonist
	Capsaicin	Agonist
	Cannabidiol	Agonist
	SOR-C13 Dexamethasone 2-APB	Inhibitor Agonist
Store-operated calcium channels	Pyr10	Inhibitor
	SKF96365	Inhibitor
	DPB-162AE/-163AE ML-9	Inhibitor/Activator
	R02959	Inhibitor
Inositol 1,4,5 triphosphate receptors ( $\text{IP}_3\text{R}$ )	Heparin, Caffeine	Inhibitor
	Xestospongin B	Inhibitor
	Xestospongin C	Inhibitor
	2-APB	Inhibitor
Voltage-Gated $\text{Ca}^{2+}$	Dihydropyridine	Inhibitor

## 7. Conclusions

$\text{Ca}^{2+}$  dysregulation is involved in the manifestation of several pulmonary diseases [48]. Because of this fact, an understanding of  $\text{Ca}^{2+}$  signaling mechanisms and the role that  $\text{Ca}^{2+}$  signaling plays in disease pathology is important. However, relatively little is known about the detailed mechanistic roles of  $\text{Ca}^{2+}$  in disease. Currently, studies have investigated the potential negative effects of vaping in relation to pulmonary disease.  $\text{Ca}^{2+}$  signaling pathways have more recently garnered attention as a potential mediator of these negative effects. These studies have demonstrated that vaping results in  $\text{Ca}^{2+}$  dysregulation, leading to decreases in cell viability and increases in inflammation. However, mechanistic details are lacking and will require further investigation. Finally, while Pyr6, Pyr10 and 2-APB were demonstrated to ameliorate disease prognosis (as described above), the mechanism is not well-understood. These data underly a need for studies to better understand the mechanism of action of vaping upon the lungs and the resultant pathology, which will include deciphering the role that  $\text{Ca}^{2+}$  dysregulation plays within this disease paradigm.

Future avenues of study will focus on further investigating the mechanistic role of  $\text{Ca}^{2+}$  signaling dysregulation within the context of diseases that might develop due to either vaping and/or tobacco smoking. Understanding the inflammatory pathways involved

via mechanistic dissection with the Ca<sup>2+</sup> channel antagonists 2-APB, Pyr10 and Pryn6 will bolster our appreciation of this field of study and potentially yield novel therapeutic targets for diseases related to Ca<sup>2+</sup> dysregulation. Furthermore, other Ca<sup>2+</sup> antagonists exist and must be investigated in relation to pulmonary disease. Another aspect of future studies will focus on investigating the role of M0 macrophage polarization to M1 macrophages as this process was recently theorized to play a role in vaping-dependent inflammatory responses.

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