Effects of chronic exposure to cigarette smoke on canonical transient receptor potential expression in rat pulmonary arterial smooth muscle

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Wang J, Chen Y, Lin C, Jia J, Tian L, Yang K, Zhao L, Lai N, Jiang Q, Sun Y, Zhong N, Ran P, Lu W. Effects of chronic exposure to cigarette smoke on canonical transient receptor potential receptor potential expression in rat pulmonary arterial smooth muscle. Am J Physiol Cell Physiol 306: C364–C373, 2014. First published December 11, 2013; doi:10.1152/ajpcell.00048.2013.—To clarify the possible mechanism of cigarette smoke (CS)-induced pulmonary hypertension and furthermore provide effective targets for prevention and treatment, the effects of chronic CS on rat pulmonary arterial smooth muscle in vivo and nicotine treatment on rat pulmonary arterial smooth muscle cells (PASMCs) in vitro were investigated. In this study, we demonstrated that chronic CS exposure led to rat weight loss, right ventricular hypertrophy, and pulmonary arterial remodeling. A fluorescence microscope was used to measure intracellular calcium concentration ([Ca2+]i) in rat distal PASMCs. Results showed that basal [Ca2+]i and store-operated calcium entry (SOCE) levels in PASMCs from 3- and 6-mo CS-exposed rats were markedly higher than those in cells from the unexposed control animals (the increases in 6-mo CS group were more significant than that in 3-mo group), accompanied with increased canonical transient receptor potential 1 (TRPC1) and TRPC6 expression at both mRNA and protein levels in isolated distal PA. Simultaneously, in vitro study showed that nicotine treatment (10 nM) significantly increased basal [Ca2+]i, and SOCE and upregulated TRPC1 and TRPC6 expression in cultured rat distal PASMCs. TRPC siRNA knockdown strategies revealed that the elevations of basal [Ca2+]i and SOCE induced by nicotine in PASMCs were TRPC1 and TRPC6 dependent. These results suggested that chronic CS-induced changes in vascular tone and structure in PA and the development of pulmonary hypertension might be largely due to upregulation of TRPC1 and TRPC6 expression in PASMCs, in which nicotine played an important role.

Recent studies showed that up to 70% of patients with COPD suffer from at least mild PH (16). Cigarette smoke (CS) has been indicated as one of the main causes of COPD and PH; studies on smokers with mild COPD demonstrated that 25% of these patients had slow or progressive increase of pulmonary arterial pressure (7, 21). Based on recent research findings, the traditional views of the pathogenesis of COPD patients with PH complications are questioned (2, 3). The traditional views believe that PH in COPD occurs as a consequence of alterations of the vasculature, such as vascular remodeling, associated with the emphysema and/or hypoxia. However, more and more evidence has shown that cigarette ingredients (such as nicotine) could directly affect the vessels and contribute to the occurrence of PH by generating a number of factors to regulate vascular contraction [i.e., endothelin-1 (ET-1)], vasodilation [i.e., endothelial nitric oxide synthase (eNOS)], vascular cell proliferation [i.e., ET-1 and vascular endothelial growth factor (VEGF)] and eventually lead to vascular remodeling and physiological responses (11, 22, 25, 33, 35–39). Research showed that CS-induced PH was essentially associated with inducible nitric oxide synthase (iNOS) and that targeting iNOS by pharmacological inhibition could improve the functional and structural abnormalities caused by CS (23). In addition, increased expression of vasoactive substances such as VEGF and ET-1 have been found in guinea pig models of chronic CS exposure, which as reported, is closely related to vascular proliferation and increased pulmonary arterial pressure (38). Canonical transient receptor potential (TRPC) channels belong to the cation channel superfamily located on the plasma membrane, which have been well defined as playing essential roles during the PH disease process through multiple mechanisms. Previously, Feng et al. (4) found that mutant worms lacking TRPC channels were defective in their response to nicotine and such a defect could be rescued by a human TRPC channel, which revealed an unexpected role of TRPC channels in regulating nicotine-dependent behavior. We and others have previously confirmed that the proteins encoded by TRPC genes are components of the store-operated calcium channel (SOCC) and receptor-operated calcium channel (ROCC) (10, 31). During PH pathophysiological progress, enhanced Ca2+ influx through SOCC, termed store-operated calcium entry (SOCE), results in elevated intracellular calcium concentration ([Ca2+]i) and subsequently promotes PASMCs migration and proliferation, leading to hypertrophy of PASMCs and increase of pulmonary vascular tone (32, 40, 41). Excessive migration and...
proliferation of PASMCs are thought to be the main reasons for pulmonary media thickness and vascular remodeling.

Based on this background, we sought to clarify whether chronic CS exposure contributes to PH development by regulating TRPC expressions and \([\text{Ca}^{2+}]_i\), in PASMCs, which are closely related to increased contractile activity and remodeling of pulmonary vessels. This study focused on investigating the effects of chronic CS exposure on TRPC1 and TRPC6 expression and SOCE in rat models of chronic CS exposure, as well as the effects of nicotine (10 nM) on \([\text{Ca}^{2+}]_i\), and TRPC expressions in cultured rat distal PASMCs to elucidate the potential mechanisms of CS-induced pulmonary hypertension.

**MATERIALS AND METHODS**

**Chronic CS exposure rat model establishment.** All procedures were approved by the Animal Care and Use Committee of Guangzhou Medical University. Adult SPF male Sprague-Dawley (SD) rats (250–300 g, provided by Experimental Animal Center of Guangdong Province) were housed in a smoke-free environment. Each exposure lasted for 1 h, and the interval between two exposures was at least 4 h; the rats were randomly grouped as control and chronic CS exposure for 1, 3, and 6 mo, respectively. The control group was housed in a smoke-free environment.

**Histology.** Heart sections of PASMCs were fixed in 4% paraformaldehyde (PFA) and dehydrated through graded concentrations of ethanol, cleared in xylene, and paraffin-embedded. Sections (5 μm) were cut with a Leica RM 2245 microtome and stained with hematoxylin and eosin (H&E). Images of the sections were taken with a Nikon Eclipse microscope (Nikon, Melville, NY).

**Real-time PCR.** Total RNA in distal pulmonary arteries or PASMCs was extracted using TRIzol (Invitrogen). mRNA was reverse transcribed in a reaction mixture of 20 μl containing 1,000 ng total RNA using iScript cDNA synthesis kit (Takara Biotechnology), followed by quantification with real-time PCR using Scafast EvaGreen superMix (Bio-Rad, Hercules, CA). The PCR reaction mixture of 25 μl was composed of 400 nM forward and reverse primers and cDNA template from 6.25 ng RNA. The program of real-time quantitative PCR consisted of a hot start at 95°C for 15 min, 45 cycles with each containing 94°C for 1 s, 57.5°C for 20 s and melting curves performed at 95°C for 1 min, 55°C for 1 min, and an increment of 0.5°C for 80 repeats. Specificity of the PCR products was sequentially verified by melting curves, agarose gel electrophoresis, and DNA sequencing. Detection threshold cycle (Ct) values were generated by iCyclerIQ software. PCR efficiency of each pair of primers was obtained from measurements of five-point serial dilutions of an unknown cDNA sample. The relative concentration of each transcript was calculated using the Pfaffl method. mRNA copies were normalized and were expressed as percent change from control values. The primers were synthesized by TAKARA Biotechnology, and the sequences are listed in Table 1.

**Western blot analysis.** The rat distal pulmonary artery was sonicated in RIPA lysis buffer (Boca Biological) with protease inhibitor cocktail tablet, and protein expression was measured by immunoblotting assay as described previously. Protein lysate was quantified using the bicinchoninic acid protein assay (Pierce, Rockford, IL). Protein lysate was then denatured by adding dithiothreitol to 150 mM and heating at 95°C for 3 min and resolved by 10% SDS-PAGE. Separated proteins were transferred onto polyvinylidene difluoride membranes (pore size 0.45 μM; Bio-Rad). Membranes were blocked with 5% nonfat dry milk in Tris-buffered saline containing 0.2% Tween 20 and incubated with affinity-purified rabbit polyclonal antibodies specific for TRPC1 (Santa Cruz Biotechnology, Santa Cruz, CA), and TRPC6 (Alomone) proteins or mouse monoclonal antibodies to α-actin (Sigma, St. Louis, MO). Bound antibodies were probed with horse-

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TRPC, canonical transient receptor potential; F, forward; R, reverse.
Effects of chronic CS exposure on RVSP, mPAP, and right ventricular hypertrophy in rats. As shown in Fig. 2, A and B, CS exposure for 1 mo and 3 mo had no significant effect on mPAP and RVSP between CS exposure and control groups. However, the mPAP in the 6-mo CS exposure group was significantly higher than that in the control (13.01 ± 0.74 vs. 9.56 ± 0.33 mmHg, P < 0.01). Also, the RVSP in the 6-mo CS exposure group was significantly elevated compared with control (29.73 ± 0.83 vs. 23.12 ± 0.837 mmHg, P < 0.01). As shown in Fig. 2C, compared with the control groups, RV/(LV + S) in the 6-mo CS exposure rats was significantly increased from 0.25 ± 0.03 to 0.31 ± 0.01 (P < 0.01), whereas in CS exposure groups for 1 and 3 mo, no significant right ventricular hypertrophy was found. These changes indicated that after 6-mo smoke exposure, the right ventricular hypertrophy was developed.

Effects of chronic CS exposure on pulmonary arterial remodeling. As shown in Fig. 3, the intima of small pulmonary arteries in rats of control groups was complete and smooth, no muscle layer thickening, and no inflammatory cell infiltration (Fig. 3, A, C, and E). In the group of 1-mo CS exposure, there were scattered inflammatory cell infiltration in the alveolar septa; the pulmonary arterial wall was not thickened compared with the control group (Fig. 3B). In group of CS exposure for 3 mo, the pulmonary arterial wall and the smooth muscle layer were observed to be thickened. Additionally, stenosis and perivascular inflammatory cell infiltration were also found (Fig. 3D). The inflammatory cell infiltration was more obvious in the 6-mo CS exposure group, in which there were marked diameter stenosis and thickening of pulmonary arterial wall (Fig. 3F). The ratio of vascular lumen area (2 x medial wall thickness/external diameter) in CS-exposed rats (average 51.94 ± 1.87%) was statistically greater than that in normal controls (average: 39.58 ± 4.23%, P = 0.001, Fig. 3G). To confirm the cell types involved in the remodeling process, endothelial cells and PASMCs on the pathological slides were labeled with anti-rat VIII factor in red and anti-rat α-actin in green (Fig. 3, H and I). There was marked diameter stenosis and thickening of smooth muscle layer in the 6-mo CS-exposed rat compared with normal control. Thickening in vascular muscular layer and thickening of smooth muscle layer in the 6-mo CS-exposed group became more apparent following the CS exposing time course.
endothelium was not detected. These results suggest that PASMCs do more direct contributions to the remodeling process than endothelial cells.

Effects of chronic exposure to CS on the SOCE and basal $[Ca^{2+}]_i$ in freshly isolated rat distal PASMCs. As shown in Fig. 4, A and B, there was no difference in SOCE between 1-mo CS exposure group and the control group; SOCE in 3-mo CS exposure group was 240.68 ± 51.49 and 151.09 ± 10.89 nM in the control group, and these results had statistical difference ($P < 0.05$); SOCE in 6-mo CS exposure group was 272.34 ± 47.62 nM, also significantly higher than that of the control group which was 180.19 ± 18.30 nM ($P < 0.01$). As shown in Fig. 4C, there was no significant difference in the basal $[Ca^{2+}]_i$ between 1-mo CS exposure group and the control group. The basal $[Ca^{2+}]_i$ in 3-mo CS exposure group was statistically higher than that in the control group (127.88 ± 12.67 vs. 107.06 ± 2.12 nM, $P < 0.05$). In 6-mo CS exposure group, the basal $[Ca^{2+}]_i$ was further elevated and exhibited significant difference compared with the control group (180.77 ± 15.17 vs. 127.65 ± 24.97 nM, $P < 0.01$).

Effects of chronic CS exposure on TRPC1 and TRPC6 mRNA and protein expression in rat distal pulmonary artery smooth muscle. As shown in Fig. 5, A and B, the CS exposure significantly promoted TRPC1 and TRPC6 mRNA expression in rat distal pulmonary artery smooth muscle, which showed a persistent increase. The most apparent upregulation occurs under 1-mo CS exposure compared with the control group, and the trend of increase slowed down in 3- and 6-mo CS exposure groups. However, significant increases were observed under all the three time points ($P < 0.01$). Figure 5, C-E, shows the effects of chronic CS exposure on TRPC1 and TRPC6 protein expression. Similarly, compared with control groups, such exposure also significantly increased TRPC1 and TRPC6 protein expression in rat pulmonary artery smooth muscle under all the three different time points (chronic CS exposure for 1, 3, and 6 mo, $P < 0.01$).

Effects of nicotine stimulation on the intracellular $Ca^{2+}$ homeostasis in cultured rat distal PASMCs. To distinguish between a direct effect of CS exposure on intracellular $Ca^{2+}$ homeostasis and the effects of circulating factors change during chronic CS exposure, we isolated the rat distal PASMCs, after appropriate culture in growth medium (3–4 days, 60–70% cell density) and starving (0.3% FBS, 24 h), cells were incubated with the main component of cigarette, nicotine (10 nM, 24 h), and PASMCs without treatment served as control. Results showed that basal $[Ca^{2+}]_i$ in the nicotine stimulated PASMCs was 136.5 ± 9.98 nM, significantly higher than that of control cells (97.67 ± 5.03 nM; Fig. 6 A, G).

Fig. 3. Effects of chronic exposure to CS on pulmonary vascular remodeling in rats ($\times$200). Lung tissues from rats under control or chronic exposure to CS for 1, 3, and 6 mo were fixed in 4% paraformaldehyde, embedded in paraffin, cross sectioned, and stained with hematoxylin and eosin (H&E staining), as shown in A, C, and E. The results are representative from 1 of 4 rats for each group. The experiment was repeated for 3 times. B, D, and F: pulmonary artery wall thickening induced by cigarette smoke exposure, compared with the control group. Arrows indicate pulmonary artery in each picture. G: difference of vascular lumen area ratio in CS-exposed rats and controls (means ± SE; n = 4). Immunostaining was performed to label the endothelial cell in red and pulmonary arterial smooth muscle cells (PASMCs) in green on the slides. Smooth muscle layer was much thickening and vascular inner diameter was dramatically stenosed in CS-exposed group. H and I: representative visual fields from normal control and CS-exposed rats.
SOCE in nicotine-stimulated cells was 315.7 ± 37.33 nM, significantly increased compared with that in control cells at 200.7 ± 17.23 nM as shown in Fig. 6, B and C (P<0.05).

**Effects of nicotine stimulation on TRPC1 and TRPC6 expression in cultured rat distal PASMCs.** In nicotine (10 nM, 24 h)-treated PASMCs, mRNA expression of TRPC1 and TRPC6 (Fig. 7A) as detected by real-time PCR was significantly increased compared with that in untreated control cells. Similarly, Western blotting analysis demonstrated that the protein levels of TRPC1 and TRPC6 (Fig. 7, B and C) were also upregulated in nicotine-treated PASMCs compared with those in untreated cells.

**Effects of TRPC1 and TRPC6 knockdown on nicotine-induced increase of basal [Ca\(^{2+}\)]_i in rat distal PASMCs.** Specific siRNA against TRPC1 (siTRPC1) and TRPC6 (siTRPC6) were synthesized, and nontargeted siRNA (siNT) was used as a control. To measure the effects of siTRPC1, 6 on nicotine upregulated TRPCs expression, cells were divided into four groups: siNT + control, siNT + nicotine, siTRPC1 + nicotine, and siTRPC6 + nicotine. siRNAs transfected cells were incubated with or without 10 nM nicotine for 24 h. The specificity and knockdown efficiency of siTRPC1 and siTRPC6 were detected using Western blotting. As shown in Fig. 8, A and B, siTRPC1 effectively reduced TRPC1 protein expression without altering TRPC6; similarly, TRPC6 expression was downregulated by siTRPC6 without significant alteration of TRPC1. Nicotine treatment increased basal [Ca\(^{2+}\)]_i (135.47 ± 3.33 nM in siNT + nicotine group vs. 108.38 ± 2.42 nM in siNT + control group, P<0.001) in PASMCs and this increase was significantly reduced after siTRPC1 and siTRPC6 treatment (117.65 ± 3.29 nM in siTRPC1 + nicotine group, and 118.67 ± 3.97 nM in siTRPC6 + nicotine group; P<0.05 each comparing to siNT + nicotine. Fig. 8E). Consistent with the results of basal [Ca\(^{2+}\)]_i, SOCE measurement revealed that nicotine treatment enhanced the SOCE (440.43 ± 20.67 nM in siNT + nicotine group, P<0.001) from baseline (187.66 ± 28.16 nM in siNT + control group). This enhancement was reduced when treated with either siTRPC1 (269.02 ± 15.29 nM in siTRPC1 + nicotine group, P<0.001) or siTRPC6 (287.12 ± 11.01 nM in siTRPC6 + nicotine group, P<0.001, Fig. 8, C and D). These results indicate that the nicotine-induced increases of basal [Ca\(^{2+}\)]_i, and SOCE in PASMC are TRPC dependent.

**DISCUSSION**

In this study, we demonstrated that chronic exposure to CS led to rat weight loss, right ventricular hypertrophy, and pulmonary arterial remodeling. For the in vivo study, chronic CS exposure for 3 and 6 mo significantly increased basal [Ca\(^{2+}\)]_i, and SOCE levels in freshly isolated rat distal PASMCs. In
association with these changes, the expression of TRPC1 and TRPC6 in rat distal PA was also found to be upregulated, suggesting the CS exposure elevated \([\text{Ca}^{2+}]_i\) is at least in part due to increased SOCE and SOCC components. Moreover, we demonstrated that nicotine treatment was also able to induce increased basal \([\text{Ca}^{2+}]_i\) and SOCE, as well as increased TRPC1 and TRPC6 expression in rat distal PASMCs.

Tobacco smoke, when burned, releases over 4,700 chemicals, and the main harmful components include carbon monoxide, nicotine, amines, nitriles, alcohols, phenols, alkanes, alkenes, carbonyl compounds, nitrogen oxides, polycyclic aromatic hydrocarbons, heterocyclic compounds, heavy metals, organic pesticides etc. Among these components, nicotine and its metabolites attract our attention because of their ability to

Fig. 5. Effects of chronic cigarette smoke exposure on canonical transient receptor potential 1 (TRPC1) and TRPC6 mRNA and protein expression in rat distal pulmonary artery smooth muscle. TRPC1 (A) and TRPC6 (B) mRNA relative to \(\beta\)-actin was measured by real-time quantitative PCR. Expression levels of TRPC1 and TRPC6 proteins were detected by Western blotting (C–E). Representative blots (C) and mean intensity for TRPC1 (D) or TRPC6 (E) blots relative to \(\alpha\)-actin. Bar values are means \(\pm\) SE (\(n = 4\) in each group). *\(P < 0.05\), significant difference from respective control group. **\(P < 0.01\), significant difference from control group.

Fig. 6. Effects of nicotine treatment (10 nM, 24 h) on the intracellular \([\text{Ca}^{2+}]_i\) homeostasis in cultured rat distal PASMCs. A: basal \([\text{Ca}^{2+}]_i\), in control and nicotine-treated PASMCs (means \(\pm\) SE; \(n = 4\)). *\(P < 0.05\), significant difference from control group. B and C: changes of SOCE in control and nicotine-treated PASMCs (means \(\pm\) SE; \(n = 4\)). *\(P < 0.05\), significant difference from control group.
stimulate PASMC proliferation, causing pulmonary artery medial hypertrophy, vascular remodeling, and luminal narrowing of blood vessels, which is thought to be a main reason resulting in PH. Wright and Churg (34) reported that the pulmonary arterial pressure of guinea pigs was increased after 6-mo exposure to CS and also the muscularization of pulmonary arteriole was enhanced. Similarly, Han et al. (6) found that CS exposure for 6 mo significantly thickened pulmonary artery medial wall and increased the RVSP in rats. Consistent with previous reports, our results in this study indicated that chronic CS exposure could induce rat pulmonary arterial wall thickness; sustained pulmonary artery thickness then leads to increase in RVSP. As a result, right ventricular hypertrophy may occur. In conclusion, chronic CS has, at least indirectly or partially, affected the right ventricle. After 3-mo CS exposure, the pulmonary arterial wall of rats started thickening, the longer...
the exposure was, the more obvious the trend was; yet, the RVSP and mean PAP were not increased until 6-mo smoke exposure.

In the rat and mouse animal models, chronic hypoxia can lead to chronic hypoxic pulmonary hypertension. We along with other groups have confirmed that chronic hypoxia contributes to the increase of [Ca^{2+}] in PASMCs, mainly caused by enhanced SOCE through SOCC (26). To demonstrate whether CS can affect SOCE in rat pulmonary arterial smooth muscle, the recovery of extracellular calcium was used. When voltage-dependent Ca^{2+} channel antagonist nifedipine was used to block the voltage-dependent Ca^{2+} channel activity, CPA rapidly elevated [Ca^{2+}], temporarily and slightly in PASMCs, suggested that calcium is released from sarcoplasmic reticulum. After the extracellular calcium concentration was recovered, CPA could make [Ca^{2+}] increased rapidly and maintained a high level; after the removal of CPA, [Ca^{2+}], returned back to the baseline level. In this process, we found that the [Ca^{2+}] in freshly isolated and short-term attached PASMCs from CS exposure rats can reach a higher marked level after recovery of extracellular calcium concentration than that in PASMCs from the control rats. These results suggest that chronic CS exposure could be an important trigger of enhanced SOCE and promote calcium influx in PASMCs.

The excitation-contraction coupling process in pulmonary vascular smooth muscle is known to be mainly dependent on the function of ion channels. The increase of [Ca^{2+}] is considered to play an essential role during the vasoconstriction and remodeling process. We linked such evidence to our previous theory about the relevant relationship among TRPC expression, intracellular calcium homeostasis, and the development of PH. It is reasonable to hypothesize that CS exposure-induced PH is potentially mediated via the regulation of the TRPC-SOCE-[Ca^{2+}] signaling cascade. SOCC is known to be mainly composed of the classical TRPC family members, among which TRPC1, TRPC4, and TRPC6 have been found to be the predominant one expressed in pulmonary artery smooth muscle of rat, mouse, and human (18, 19, 30–32). The upregulation of TRPC expression can increase the activity of SOCC, relating to enhanced SOCE, and also promotes the proliferation of PASMCs (5, 42). Additionally, upregulated TRPC1 and TRPC6 expression and elevated SOCE are also observed under chronic hypoxia in rat distal PASMCs (9, 32). In the present study, similar to the effects of chronic hypoxia, chronic exposure to CS promotes the [Ca^{2+}], and SOCE, as well as upregulated TRPC1 and TRPC6 expression in PASMCs. These results suggest that upregulation of TRPC1 and TRPC6 expression could be a key element attributable to the enhanced basal [Ca^{2+}] and SOCE activity during chronic CS exposure. Besides TRPCs, other Ca^{2+}-related molecules such as STIM and Orai (13, 20), as well as the ability of the cells to maintain intracellular Ca^{2+} homeostasis depending on the mitochondrial function, ATP storage, and cell membrane permeability (8, 27), were also considered to be able to affect the intracellular calcium changes. It is, therefore, not surprising to note that TRPC proteins in rat distal PA were upregulated as early as 1-mo exposure to CS, whereas the changes in the Ca^{2+} level were not detectable until 3-mo exposure to CS. Further study is needed to elucidate the molecular mechanisms underlying the delayed calcium response following changes of TRPC1 and TRPC6 expression in PA smooth muscle.

Subsequently, additional experiments with nicotine treatment in vitro were performed to elucidate whether nicotine was responsible for the upregulation of TRPC1, TRPC6 expression, and the increases of [Ca^{2+}], and SOCE in PASMCs. Consistent with the results from chronically CS exposed rat, nicotine treatment (10 nM, 24 h) significantly increased the basal [Ca^{2+}], and SOCE levels in cultured rat distal PASMCs. TRPC1 and TRPC6 expression in PASMCs was observed to be upregulated by nicotine, and specific knockdown of TRPC1 and TRPC6 by siRNAs revealed that the nicotine-induced increases of basal [Ca^{2+}] and SOCE in PASMCs were TRPC dependent. In previous studies, nicotine has been proven to exert biological effects through binding to nicotinic acetylcholine receptors (nAChRs), which then induced activation of related signal transduction pathways, including Ca^{2+} influx and activation of calmodulin, protein kinase C, c-Src, and so on. nAChRs belong to the Pentamers formed by 12 kinds of subunits (α2–10 and β2–4) (28). Other studies have shown that there is a higher affinity and density of nicotinic acetylcholine receptors in the brain of smokers than nonsmokers (17). Intake of nicotine to pregnant rhesus monkeys made the α7-nAChR subunit expression levels increase in lung tissue of the fetus significantly and the maturation of the lung was also affected (24). Similar to mammals, nicotine can act on the nAChR of A Caenorhabditis elegans (a worm), leading to acute nicotine response, tolerance, withdrawal, and excitement and so on. However, mutant worms lack of TRPC channels are defective in their response to nicotine and such a defect can be rescued by a human TRPC channel. Therefore, it is likely that the alterations of TRPC and the basal [Ca^{2+}], and SOCE levels are nicotine dependent and might be the result of nAChR modulation on the surface of rat distal PASMCs. However, researchers recently demonstrated that a metabolic product of CS can change the epigenetic modifications of DNA, including promoter methylation profiles of critical genes involved in lung cancer (29). CS has also been found to affect the stability of noncoding RNAs (e.g., microRNA) that regulate message stability (15). Collectively, it cannot be excluded that CS exposure could cause changes in TRPC1 and TRPC6 expression directly at transcriptional or posttranscriptional level, or perhaps, via other pathways. Finally, the exact mechanism of long-term CS exposure-induced pulmonary vascular remodeling still is largely unknown and awaits further studies.

In conclusion, we found that chronic CS exposure could significantly promote TRPC1, TRPC6 mRNA, and protein expression and increase SOCE activity and basal [Ca^{2+}], in PASMCs. In vitro experiments suggested that the effects of nicotine on TRPC expression and intracellular calcium homeostasis of distal PASMCs likely play an indispensable role during the development of CS-induced PH.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).
AUTHOR CONTRIBUTIONS


REFERENCES

