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Nicotinic α7 Acetylcholine Receptor (α7nAChR) in Human Airway Smooth Muscle

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Abstract

Diseases such as asthma are exacerbated by inflammation, cigarette smoke and even nicotine delivery devices such as e-cigarettes. However, there is currently little information on how nicotine affects airways, particularly in humans, and changes in the context of inflammation or asthma. Here, a longstanding assumption is that airway smooth muscle (ASM) that is key to bronchoconstriction has muscarinic receptors while nicotinic receptors (nAChRs) are only on airway neurons. In this study, we tested the hypothesis that human ASM expresses α7nAChR and explored its profile in inflammation and asthma using ASM of non-asthmatics vs. mild-moderate asthmatics. mRNA and western analysis showed the α7 subunit is most expressed in ASM cells and further increased in asthmatics and smokers, or by exposure to nicotine, cigarette smoke or pro-inflammatory cytokines TNFα and IL-13. In these effects, signaling pathways relevant to asthma such as NFκB, AP-1 and CREB are involved. These novel data demonstrate the expression of α7nAChR in human ASM and suggest their potential role in asthma pathophysiology in the context of nicotine exposure.

Keywords

asthma; inflammation; cigarette smoke; nicotinic receptors

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Introduction

Airway smooth muscle (ASM) cells play a critical role in bronchial structure and function via their role in regulation of contractility and remodeling including proliferation and extracellular matrix production in the context of diseases such as asthma and COPD (Amrani et al., 2020; Black, Panettieri, Banerjee, & Berger, 2012; M. Lam, Lamanna, & Bourke, 2019; Lo, Kennedy, Kurten, Panettieri, & Koziol-White, 2018; Prakash, 2013, 2016). Factors such as cigarette smoke are known to exacerbate asthma and contribute to COPD (Beasley, Semprini, & Mitchell, 2015; Burney, Jarvis, & Perez-Padilla, 2015; Ellis & Soliman, 2015; Gibson-Young, Martinasek, Tamulevicius, Fortner, & Alanazi, 2020; Lundback, Backman, Lotvall, & Ronmark, 2016; May & Li, 2015; Postma, Bush, & van den Berge, 2015; Sheehan & Phipatanakul, 2015; St Claire et al., 2020; Traboulsi et al., 2020; Viegi, Maio, Pistelli, Baldacci, & Carrozzi, 2006; Wurst, Kelly-Reif, Bushnell, Pascoe, & Barnes, 2016). However, the mechanisms by which CS affects ASM *per se* in airway hyperresponsiveness are still under investigation (Aravamudan et al., 2014; Sathish et al., 2015; Vogel et al., 2014; Wylam et al., 2015; Zuo et al., 2018).

There is increasing recognition that nicotine, the major active element of CS, contributes to increased airway hyperresponsiveness (Chun, Moazed, Calfee, Matthay, & Gotts, 2017; Clapp & Jaspers, 2017; Gibson-Young et al., 2020; Jankowski, Brozek, Lawson, Skoczynski, & Zejda, 2017; St Claire et al., 2020; Traboulsi et al., 2020). Such effects of nicotine are relevant to both smoking and nicotine delivery devices such as e-cigarettes. However, the mechanisms by which nicotine affects ASM or how such effects change in asthma or inflammation, are not well-understood. Previous studies in rat ASM have found nicotine elevates intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) (Hong & Lee, 1996; Jiang et al., 2014). Classically, nicotine acts through nicotinic acetylcholine receptors (nAChRs). However, in ASM, the common assumption is that contractility involves muscarinic receptors. Less is known regarding expression or functionality of nAChR in ASM. Limited data show α7nAChR is expressed in rat ASM (He et al., 2014; Hong & Lee, 1996; Jiang et al., 2014). Whether human ASM expresses nAChR subunits is not known, nor is the effect of inflammation, asthma or CS on nAChR portfolios that could contribute to observed effects of nicotine or CS on the airway. Therefore, the present study was designed to explore nAChR in human ASM with the hypothesis that human ASM expresses α7nAChR and its expression is increased in inflammation and asthma, providing mechanistic basis for nicotine effects in the airway.

Materials and Methods

Reagents and supplies:

Cell culture reagents and supplies including Dulbecco's Modified Eagle's Medium/F12 (DMEM/F12), trypsin and Antibiotic-Antimycotic (AbAm) were purchased from Invitrogen (Carlsbad, CA). Charcoal stripped fetal bovine serum (FBS) was from Sigma-Aldrich (St. Louis, MO). α7nAChR antibody was from Novus Biologicals (Littleton, CO). Other antibodies for α4nAChR, β2nAChR, caldesmon, α-SMA, calponin-1 and transgelin/SM-22 were obtained from Abcam (Cambridge, UK). β-actin antibody was from ABM Biological Materials (Richmond, Canada). Pro-inflammatory cytokines, tumor necrosis factor alpha

(TNFα) and interleukin-13 (IL-13) were from R&D Systems (Minneapolis, MN). RIPA cell lysis/extraction buffer and A/G PLUS Agarose beads for co-immunoprecipitation techniques were obtained from Thermo Fisher Scientific (Waltham, MA) and SantaCruz Biotechnology (Dallas, TX) respectively. Pharmacological inhibitors for signaling pathways NFkB (SN-50) and STAT6 (AS1517499) were from SantaCruz Biotechnology (Dallas, TX) and Millipore Sigma (Burlington, MA) respectively. STAT3 (SD 1008), AP1 (SR 11302), CREB (666-15) and MAPK (PD 98059) were from Tocris Bioscience (Bristol, UK). Fluorescent secondary antibodies were from Li-Cor Biosciences (Lincoln, NE). Other chemicals/drugs/antibodies were from Sigma-Aldrich unless otherwise specified.

Primary human ASM cells:

A previously described technique (Aravamudan et al., 2014; Sathish et al., 2014) was followed to isolate human ASM cells from lung samples incidental to patient thoracic surgery at Mayo Clinic, Rochester, MN (approved by the Mayo Clinic Institutional Review Board and considered minimal risk). Briefly, third to sixth generation bronchi were isolated from lung resections for non-infectious indications including focal cancers (bronchoalveolar carcinoma was excluded). Asthma diagnoses and severity was based on standard clinical criteria including pulmonary function testing, as noted in patients' clinical records. Non-asthmatics were defined as patients with no noted history of lung disease and were clinically deemed to have normal lung function. Samples were from both male and female adults ranging from 21 to 80 years of age. ASM was dissected from healthy appearing lung areas identified with the help of a pathologist. Samples were enzymatically dissociated as previously described (Aravamudan et al., 2014; Sathish et al., 2014) to isolate ASM cells, which were placed in cell culture under standard conditions of 37°C (5% CO_2, 95% air) using DMEM/F12 supplemented with 10% FBS and 1% AbAm. Cells <5 passages of subculture were serum-deprived for a minimum of 24 hours prior to all experimental procedures. Furthermore, human ASM cell phenotype validations were also performed periodically (Supplement Figure 1).

Cell Treatment:

Following serum starvation, cells were treated with TNFα (20ng/ml) or IL-13 (50ng/ml) in the presence/absence of inhibitors for 24 h. For signaling studies, non-asthmatic ASM cells were treated with pharmacological inhibitors 2h prior to addition of TNFα or IL-13 for another 24h. Expression was assessed at protein and mRNA levels.

qRT-PCR:

Cells were washed with RNA-grade DPBS, trypsinized and centrifuged. Total RNA was extracted from cells using Quick-RNA™ MiniPrep kit (Zymo Research, Irvine, CA) following manufacturer's protocol and complementary DNA was synthesized using OneScript cDNA Synthesis Kit (Richmond, BC, Canada). Standard qPCR techniques were followed (optimized for Roche LC480 Light Cycler) using QuantStudio 3 RT-PCR system as per the manufacturer's instructions. The following primers obtained from Integrated DNA Technologies (Coralville, IA) were used for qRT-PCR analysis: α7nAChR (forward 5'-CAA TGG AGA ATG GGA CCT AGT G-3', reverse 5'-GCA GCA TGA AGA CGG TAA GA-3'), s16 (forward 5′-CAA TGG TCT CAT CAA GGT GAA CGG-3′, reverse 5′-CTG

GAT AGC ATA AAT CTG GGC-3′). Fold changes in mRNA expression were calculated by normalization of cycle threshold [C(t)] value of target genes to reference gene S16 using the ΔΔCt method.

Immunoblotting:

ASM cell lysates were prepared using cell lysis buffer (Cell Signaling Technologies, Beverly, MA) containing protease and phosphatase inhibitors using previously described methods (Aravamudan et al., 2014; Sathish et al., 2014). Resultant supernatants were assayed for total protein content using the DC protein Assay kit (BioRad, Hercules, CA, USA). Approximately 30 μg of each lysate were loaded on 10% SDS-page and transferred onto 0.22 μm PVDF membranes. Non-specific binding was blocked using 5.0% bovine serum albumin (BSA) and membranes probed overnight at 4°C with antibodies of interest. Blots were then incubated with Li-Cor secondary antibodies. Protein expression detection and densitometry were performed on a Li-Cor Odyssey IR scanning system (Lincoln, NE). Band intensities were normalized against β-actin.

Co-immunoprecipitation (Co-IP) of α4nAChR, α7nAChR and β2nAChR:

Co-IP assays were performed according to previously published protocols (Sathish, Xu, Karmazyn, Sims, & Narayanan, 2006; Townsend, Sathish, Thompson, Pabelick, & Prakash, 2012). Briefly, fully confluent human ASM cells were washed twice in ice-cold DPBS. After washing, cells were scraped off directly with a cell scraper using 1X RIPA Lysis and Extraction Buffer. Cells were then centrifuged at 14,000 rpm for 10 min, and the supernatant was collected followed by protein estimation. For α4nAChR pull-down assays, A/G PLUS–agarose beads (Santa Cruz Biotechnology) were incubated with 2% BSA in DPBS for 2 h at room temperature. Post incubation with BSA, A/G PLUS agarose beads were vortexed for 30 sec and centrifuged in a Beckmann centrifuge for 10 min at 14,000 rpm to remove residual BSA. Then, BSA-treated A/G Plus agarose beads were incubated with 0.2 μg primary antibody (α4nAChR) by gentle rocking for 30 min at room temperature. Then agarose-bound α4nAChR was added to each protein sample (via RIPA extraction as above) at a concentration of 1mg/mL with gentle rocking overnight at 4°C. The sample mixtures containing protein sample, A/G PLUS agarose beads and α4nAChR antibody were vortexed for 30 sec and centrifuged for 30 min at 14,000 rpm. The mixtures were washed five times with cold washing buffer (20mM HEPES (pH 7.5), 150mM NaCl, 1mM EDTA and 0.5% Tween-20 in DPBS) to remove unbound protein. The sample mixtures were then denatured with 1X SDS sample buffer before loading in a 4-20% polyacrylamide gel. After blocking with non-fat milk suspension, the PVDF membranes were probed for α7nAChR or β2nAChR overnight at 4°C, followed by the Li-Cor Odyssey CLx Imaging detection using a Li-Cor IRDye secondary antibodies (Lincoln, NE).

Immunofluorescence:

ASM cells were fixed in 4% paraformaldehyde for 15 min, washed in DPBS, blocked with 4% normal donkey serum, and exposed overnight to rabbit anti-α7nAChR antibody. Specificity of the antibody to α7nAChR was tested by pre-exposure of living ASM cells to alpha-bungarotoxin (αBTX; List Biologicals) prior to fixation. Cells were then washed with PBS and exposed to Cy3-conjugated donkey anti-rabbit secondary antibody. Nuclei were

counterstained with DAPI. Stained samples were imaged on a Nikon Ti-U inverted microscope with a 40X/1.3 objective lens and epifluorescence attachments.

Statistical analysis:

All experiments were performed in human ASM cells from at least 3 patients (non-asthmatic/asthmatic) and performed in duplicate for each patient sample for qRT-PCR and western analyses. Statistical comparisons were made using either one-way ANOVA or two-way ANOVA as appropriate, followed by Bonferroni *post-hoc* multiple comparisons test using GraphPad Prism version 8.0.0 for Windows, GraphPad Software, San Diego, California USA, www.graphpad.com. Statistical significance was tested at a level of $p<0.05$. All values are expressed as means ± SEM. "N" values representing numbers of patients' samples in the figure legends.

Results

α4nAChR and α7nAChR are expressed in human ASM

ASM cells from non-asthmatic patients were processed for mRNA-based portfolio of nAChR subunits known to be expressed in humans (Changeux & Taly, 2008). Interestingly, only α4nAChR and α7nAChR showed substantial expression with much smaller expression of the β2 subunit (Fig. 1A). In comparison, other subunits were minimally expressed. Based on these data, we further focused only on the α4 and α7 subunits. Interestingly, asthmatic ASM showed significantly decreased expression of α4nAChR protein (Fig. 2A). Furthermore, in cells exposed to TNFα or IL-13, there was no significant change in α4nAChR levels, with a trend towards small increase with either cytokine (Fig 2B). In contrast, asthmatic ASM showed significantly increased expression of α7nAChR mRNA (Fig. 3A) and protein (Fig. 3B). Immunofluorescence staining of ASM cells showed plasma membrane localization of α7nAChR (Fig 1B) absent in cells pre-exposed to αBTX. Furthermore, protein expression of α7nAChR was significantly elevated in patient samples from smokers compared to non-smokers (Fig. 4). Accordingly, subsequent studies focused on α7nAChR.

Cytokine-induced changes in α7nAChR in human ASM

We next examined if cytokines affected expression of α7nAChR, and differences between non-asthmatic vs. asthmatic ASM. Therefore, ASM cells were exposed to TNFα (20ng/ml) or IL-13 (50ng/ml) for 24h and harvested at 6 h, 12 h and 24 h time intervals. qRT-PCR showed that expression of α7nAChR was increased in the presence of either cytokine in non-asthmatics (Fig. 5) but significantly only at 24 h (Fig 5E). In comparison, there were significant increases in α7nAChR mRNA expression at all three time-points for asthmatic ASM (Fig. 5B, D, F). Interestingly, IL-13 appeared to have a more pronounced effect in comparison to TNFα particularly in asthmatics. This was not necessarily reflected by protein expression which nonetheless showed increased α7nAChR in non-asthmatic ASM with both cytokines compared to vehicle control (Fig 6A). Interestingly, 1% or 2% cigarette smoke extract (CSE) also resulted in increased α7nAChR (Fig 6B).

Mechanisms of α7nAChR regulation *in human ASM*

We examined the role of transcriptional regulation of α7nAChR, focusing on STAT6, NFκB, and AP-1 relevant to airway inflammation, using pharmacological inhibitors given 2 h before exposure to either TNFα or IL-13 for 24 h. Interestingly, STAT6 inhibition (20 nM AS151749) significantly reduced IL-13 effects on α7nAChR but not TNFα (Fig 7A). Pretreatment with an NFκB inhibitor (SN50, 20 μM) or AP-1 inhibitor (SR1302; 1 μM) significantly blunted α7nAChR enhancement by either cytokine (Fig 7B and Fig 5C).

To determine the role of major cytosolic signaling pathways, we focused on the ERK pathway using a MAPK inhibitor (PD98059; 2 μM). Interestingly, we found no significant effects of this inhibitor on either TNFα or IL-13-induced increase in α7nAChR expression (Fig 8A). CREB is an important cytoplasmic-nuclear trafficking molecule in the transcriptional machinery of ASM (Prakash, 2016). Interestingly, only TNFα-induced increase in α7nAChR expression was blunted on pharmacological inhibition of CREB (Fig. 8B).

To investigate interactions amongst α4, α7 and β2 receptor subunits, we performed Co-IP experiments which showed that both α7nAChR and β2nAChR interact with α4nAChR to some extent in human ASM cells. However, this colocalization was not significantly different between non-asthmatics and asthmatics (Fig. 9).

Discussion

Inflammation in asthma enhances ASM Ca^{2+} and contractility response to bronchoconstrictor agonists such as ACh (Guedes et al., 2015; Koopmans et al., 2014; Prakash, 2013, 2016; Wright et al., 2013). ACh effects on ASM are usually associated with muscarinic receptors, while nAChRs are generally considered to act as Ca^{2+} channels in preganglionic neurons (Belmonte, 2005; Canning, 2006; Canning & Fischer, 2001; Pieper, 2012; Racke & Matthiesen, 2004; Rogers, 2002; van der Velden & Hulsmann, 1999). However, in the context of CS exposure as well as nicotine delivery devices such as e-cigarettes, it is important to consider whether and how inhaled nicotine influences airway cells such as epithelium and ASM. Clinical data shows that CS exacerbates asthma (Beasley et al., 2015; Burney et al., 2015; Ellis & Soliman, 2015; Lundback et al., 2016; May & Li, 2015; Postma et al., 2015; Sheehan & Phipatanakul, 2015; Wurst et al., 2016) and that e-cigarettes while seen as friendly for asthmatics (Lucchiari et al., 2016; Meo & Al Asiri, 2014; Oh & Kacker, 2014; Orellana-Barrios, Payne, Mulkey, & Nugent, 2015; Rowell & Tarran, 2015; Walton et al., 2015) may in fact not be as benign for asthmatics (Hsieh, 2016; Oh & Kacker, 2014) and are increasingly seen as toxic (Chun et al., 2017; Clapp & Jaspers, 2017; Jankowski et al., 2017; Javed, Kellesarian, Sundar, Romanos, & Rahman, 2017; G. Kaur, Muthumalage, & Rahman, 2018; McEvoy & Spindel, 2017). Nicotine also remains relevant as long as smoking exists. However, the mechanisms of nicotine action even in normal airways are surprisingly understudied, and barely understood in asthmatic airways. Furthermore, little is known about nAChR expression and roles in human ASM, inflammation or asthma. The present data show that human ASM in fact do express nAChR, largely α7nAChR. Furthermore, α7nAChR expression is increased with exposure to cigarette smoke or asthma-relevant cytokines such as TNFα or IL-13, and importantly in

asthmatics and smokers. Given the roles of ASM in airway hyperreactivity (Guedes et al., 2015; Koopmans et al., 2014; Prakash, 2013, 2016; Wright et al., 2013) and remodeling, (Berair & Brightling, 2014; Guedes et al., 2015; Hirota & Martin, 2013; Noble et al., 2014; Prakash, 2013, 2016) these findings on α7nAChR become important.

Nicotine acts on nAChRs of airway-related vagal preganglionic neurons in the central nervous system (Dehkordi, Kc, Balan, & Haxhiu, 2006; Q. Gu, Ni, & Lee, 2008; Krasteva et al., 2011; Lee et al., 2007; Lee & Gu, 2009). Cholinergic nerves enhance bronchoconstriction, mucus secretion, and bronchial vasodilation (Belmonte, 2005; Canning, 2006; Canning & Fischer, 2001; Racke & Matthiesen, 2004; Richardson & Peatfield, 1987), but abnormal cholinergic innervation in asthma has not been definitively proven. Nicotine absorption in blood (Schneider, Lunell, Olmstead, & Fagerstrom, 1996; Schneider, Olmstead, Franzon, & Lunell, 2001) could influence airway innervation, but inhaled nicotine certainly has the potential to directly target airway epithelium, ASM and fibroblasts: cell types that play critical roles in asthma (Davies, 2014; Loxham, Davies, & Blume, 2014; Pain et al., 2014; Parulekar, Atik, & Hanania, 2014; Prakash, 2013). Yet, effects of inhaled nicotine or even mechanisms of nicotine action via nAChRs on normal resident cells of the airway, particularly ASM are not well-studied (Hahn, Lang, Bleicher, Zwerenz, & Rausch, 1992; Hartiala, Mapp, Mitchell, & Gold, 1985; He et al., 2014; Hong & Lee, 1996; Jiang et al., 2014). Here, this likely reflects assumptions that nAChRs are involved in airway reflexes, while ASM have only mAChRs (Belmonte, 2005; Canning, 2006; Canning & Fischer, 2001; Pieper, 2012; Racke & Matthiesen, 2004; Rogers, 2002; van der Velden & Hulsmann, 1999). In this regard, there is also no information on how nicotine influences asthmatic airways, or whether/how inflammation alters nAChR expression and signaling in asthma. The present study provides initial evidence for nAChR expression in human ASM, showing increased expression in asthmatics and smokers, as well as with pro-inflammatory cytokines that could contribute to increased cholinergic responses directly at the level of the ASM.

There are 16 nAChR subunits (α1–7, α9–10, β1–4, δ, ε, γ) with different pentameric configurations (Changeux & Taly, 2008; Fasoli & Gotti, 2015; Leonard & Bertrand, 2001; Zoli, Pistillo, & Gotti, 2015), but “muscle-type” subunits (α1, β1, δ, ε, γ) known in skeletal muscle are unlikely to be involved in the airway. Indeed, our data in human ASM show no mRNA expression for these subunits. Among “neuronal” subunits, α7 and α9 form homopentameric nAChRs, and importantly, α7nAChRs show high Ca^{2+} permeability (Leonard & Bertrand, 2001), and are thus appealing in the context of airway reactivity. α7nAChR is expressed in neurons (Albuquerque et al., 2000), epithelium (Y. Wang et al., 2001), endothelium (Y. Wang et al., 2001), macrophages (H. Wang et al., 2003), and arterial smooth muscle (Bruggmann, Lips, Pfeil, Haberberger, & Kummer, 2002). In epithelium, α4, α5 and α7 levels correlate with lung function (Prakash, 2016), but nicotine enhances only α7. There are limited data on nAChR profiles in ASM with rat ASM showing high α7nAChR (Jiang et al., 2014). In this regard, our data showing largely α7nAChR in the human ASM is consistent. Here, we have used highly specific αBTX for detection of surface α7nAChR in human ASM cells. We further show α4nAChR expression, albeit much lower compared to α7nAChR. Thus, in terms of human ASM, it is likely that α7nAChR and perhaps to a lesser extent α4nAChR are the key subunits that may be important towards

functional cholinergic or nicotinic effects. Our Co-IP findings suggest that there is an interaction of α4 with α7 and β2 subunits. These interactions may be functionally important for α7-mediated effects of nicotine in asthma. However, such functional effects remain to be investigated.

We find that α7nAChR is substantially increased by TNFα and IL-13: cytokines relevant to asthma (Lerner et al., 2015; Lin et al., 2010; Rincon & Irvin, 2012; Svenningsen & Nair, 2017), and known to affect ASM (Ammit et al., 2007; De, Zelazny, Souhrada, & Souhrada, 1995; Delmotte & Sieck, 2015; Guedes et al., 2015; Jia et al., 2013; D. Kaur et al., 2015; Matsumoto et al., 2012; Robinson et al., 2015; Sathish et al., 2014). We further find that α7nAChR is increased in ASM from both asthmatics and smokers. Thus, regardless of what function α7nAChR may have in ASM, such effects are likely to be exacerbated in inflammation relevant to both asthma and cigarette smoke exposure. In contrast, α4nAChR expression is not substantially altered in asthmatics and only slightly enhanced by cytokines. Whether this is functionally important remains to be determined. Thus, at least on the human ASM cellular level, α7nAChR may be particularly important.

How upstream regulation of ASM α7nAChR occurs with inflammation or in asthma has not been previously explored. This is an important aspect since intracellular α7nAChR is non-responsive to nicotine and presumably ACh unless nAChR- and α7-specific ER chaperones “resistance to inhibitor of cholinesterase” (RIC-3) (Ben-David et al., 2016; Ben-David & Treinin, 2017; Cohen Ben-Ami et al., 2009; Millar, 2008) and NACHO (S. Gu et al., 2016; Matta et al., 2017; Rex, Shukla, Gu, Bredt, & DiSepio, 2017) are expressed, and increase plasma membrane levels of the receptor. Ongoing studies in human ASM do show RIC-3 and NACHO (Borkar, Sathish, Pabelick, unpublished observations). Nonetheless, our immunofluorescence studies do show plasma membrane expression of α7nAChR suggesting functional receptor levels (although we recognize there is likely substantial intracellular expression as well). Whether such plasma membrane α7nAChR or the associated chaperones are increased in inflammation or asthma remains to be determined. Regardless, our studies do find that cytokines can regulate α7nAChR expression via multiple signaling pathways all relevant to airway inflammation (Berair & Brightling, 2014; Berair, Saunders, & Brightling, 2013; Hirota & Martin, 2013; Manuyakorn, Howarth, & Holgate, 2013; Noble et al., 2014; Prakash, 2013, 2016). Here, pathways such as NFkB and AP-1 (relevant to TNF), STAT6 (relevant to IL-13) and CREB appear to be important. Interestingly these pathways are also downstream effectors of nicotine at least in immune cells (Bregeon et al., 2011; Gahring, Myers, Dunn, Weiss, & Rogers, 2017; D. C. Lam et al., 2016; Narumoto et al., 2010; Narumoto et al., 2013; Sitapara et al., 2014; Su, Matthay, & Malik, 2010). Thus, it is possible that α7nAChR activation in airways promotes inflammatory signaling. Indeed, nicotine has been shown to have pro-proliferative, pro-inflammatory and anti-apoptotic activity in rat ASM (He et al., 2014).

In summary, the present study shows that α7nAChR is the major nicotinic receptor expressed in human ASM, with its expression significantly increased in asthmatics and smokers, as well as with inflammation. Thus, α7nAChR may play a key role in ASM hyperresponsiveness and other functional changes that occur in asthma and with nicotine or cigarette smoke exposure.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

Albuquerque EX, Pereira EF, Mike A, Eisenberg HM, Maelicke A, & Alkondon M (2000). Neuronal nicotinic receptors in synaptic functions in humans and rats: physiological and clinical relevance. Behavioural brain research, 113(1-2), 131–141. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10942040 [PubMed: 10942040]

Ammit AJ, Moir LM, Oliver BG, Hughes JM, Alkhouri H, Ge Q, … Roth M (2007). Effect of IL-6 trans-signaling on the pro-remodeling phenotype of airway smooth muscle. American journal of physiology. Lung cellular and molecular physiology, 292(1), L199–206. doi:10.1152/ajplung.00230.2006 [PubMed: 16936245]

Amrani Y, Panettieri RA, Ramos-Ramirez P, Schaafsma D, Kaczmarek K, & Tliba O (2020). Important lessons learned from studies on the pharmacology of glucocorticoids in human airway smooth muscle cells: Too much of a good thing may be a problem. Pharmacol Ther, 213, 107589. doi:10.1016/j.pharmthera.2020.107589 [PubMed: 32473159]

Aravamudan B, Kiel A, Freeman M, Delmotte P, Thompson M, Vassallo R, … Prakash YS (2014). Cigarette smoke-induced mitochondrial fragmentation and dysfunction in human airway smooth muscle. American journal of physiology. Lung cellular and molecular physiology, 306(9), L840–854. doi:10.1152/ajplung.00155.2013 [PubMed: 24610934]

Beasley R, Semprini A, & Mitchell EA (2015). Risk factors for asthma: is prevention possible? Lancet, 386(9998), 1075–1085. doi:10.1016/S0140-6736(15)00156-7 [PubMed: 26382999]

Belmonte KE (2005). Cholinergic pathways in the lungs and anticholinergic therapy for chronic obstructive pulmonary disease. Proc Am Thorac Soc, 2(4), 297–304; discussion 311-292. doi:10.1513/pats.200504-043SR [PubMed: 16267352]

Ben-David Y, Mizrachi T, Kagan S, Krisher T, Cohen E, Brenner T, & Treinin M (2016). RIC-3 expression and splicing regulate nAChR functional expression. Mol Brain, 9(1), 47. doi:10.1186/s13041-016-0231-5 [PubMed: 27129882]

Ben-David Y, & Treinin M (2017). Regulation of RIC-3 and of nAChR expression. Oncotarget, 8(4), 5662–5663. doi:10.18632/oncotarget.13925 [PubMed: 28086205]

Berair R, & Brightling CE (2014). Asthma therapy and its effect on airway remodelling. Drugs, 74(12), 1345–1369. doi:10.1007/s40265-014-0250-4 [PubMed: 25056652]

Berair R, Saunders R, & Brightling CE (2013). Origins of increased airway smooth muscle mass in asthma. BMC medicine, 11, 145. doi:10.1186/1741-7015-11-145 [PubMed: 23742314]

Black JL, Panettieri RA Jr., Banerjee A, & Berger P (2012). Airway smooth muscle in asthma: just a target for bronchodilation? Clin Chest Med, 33(3), 543–558. doi:10.1016/j.ccm.2012.05.002 [PubMed: 22929101]

Bregeon F, Xeridat F, Andreotti N, Lepidi H, Delpierre S, Roch A, … Steinberg JG (2011). Activation of nicotinic cholinergic receptors prevents ventilator-induced lung injury in rats. PloS one, 6(8), e22386. doi:10.1371/journal.pone.0022386 [PubMed: 21857926]

Bruggmann D, Lips KS, Pfeil U, Haberberger RV, & Kummer W (2002). Multiple nicotinic acetylcholine receptor alpha-subunits are expressed in the arterial system of the rat. Histochemistry and cell biology, 118(6), 441–447. doi:10.1007/s00418-002-0475-2 [PubMed: 12483309]

Burney P, Jarvis D, & Perez-Padilla R (2015). The global burden of chronic respiratory disease in adults. The international journal of tuberculosis and lung disease : the official journal of the International Union against Tuberculosis and Lung Disease, 19(1), 10–20. doi:10.5588/ijtld.14.0446

Canning BJ (2006). Reflex regulation of airway smooth muscle tone. J Appl Physiol (1985), 101(3), 971–985. doi:10.1152/japplphysiol.00313.2006 [PubMed: 16728519]

Canning BJ, & Fischer A (2001). Neural regulation of airway smooth muscle tone. Respir Physiol, 125(1-2), 113–127. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/11240156 [PubMed: 11240156]

Changeux JP, & Taly A (2008). Nicotinic receptors, allosteric proteins and medicine. Trends in molecular medicine, 14(3), 93–102. doi:10.1016/j.molmed.2008.01.001 [PubMed: 18262468]

Chun LF, Moazed F, Calfee CS, Matthay MA, & Gotts JE (2017). Pulmonary Toxicity of E-cigarettes. American journal of physiology. Lung cellular and molecular physiology, ajplung 00071 02017. doi:10.1152/ajplung.00071.2017

Clapp PW, & Jaspers I (2017). Electronic Cigarettes: Their Constituents and Potential Links to Asthma. Curr Allergy Asthma Rep, 17(11), 79. doi:10.1007/s11882-017-0747-5 [PubMed: 28983782]

Cohen Ben-Ami H, Biala Y, Farah H, Elishevitz E, Battat E, & Treinin M (2009). Receptor and subunit specific interactions of RIC-3 with nicotinic acetylcholine receptors. Biochemistry, 48(51), 12329–12336. doi:10.1021/bi901234a [PubMed: 19899809]

Davies DE (2014). Epithelial barrier function and immunity in asthma. Annals of the American Thoracic Society, 11 Suppl 5, S244–251. doi:10.1513/AnnalsATS.201407-304AW [PubMed: 25525727]

De S, Zelazny ET, Souhrada JF, & Souhrada M (1995). IL-1 beta and IL-6 induce hyperplasia and hypertrophy of cultured guinea pig airway smooth muscle cells. J Appl Physiol (1985), 78(4), 1555–1563. doi:10.1152/jappl.1995.78.4.1555 [PubMed: 7615469]

Dehkordi O, Kc P, Balan KV, & Haxhiu MA (2006). Airway-related vagal preganglionic neurons express multiple nicotinic acetylcholine receptor subunits. Autonomic neuroscience : basic & clinical, 128(1-2), 53–63. doi:10.1016/j.autneu.2006.02.023 [PubMed: 16616705]

Delmotte P, & Sieck GC (2015). Interaction between endoplasmic/sarcoplasmic reticulum stress (ER/SR stress), mitochondrial signaling and Ca(2+) regulation in airway smooth muscle (ASM). Canadian journal of physiology and pharmacology, 93(2), 97–110. doi:10.1139/cjpp-2014-0361 [PubMed: 25506723]

Ellis AK, & Soliman M (2015). Year in review: upper respiratory diseases. Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology, 114(3), 168–169. doi:10.1016/j.anai.2014.12.011

Fasoli F, & Gotti C (2015). Structure of neuronal nicotinic receptors. Current topics in behavioral neurosciences, 23, 1–17. doi:10.1007/978-3-319-13665-3_1 [PubMed: 25655885]

Gahring LC, Myers EJ, Dunn DM, Weiss RB, & Rogers SW (2017). Nicotinic alpha 7 receptor expression and modulation of the lung epithelial response to lipopolysaccharide. PloS one, 12(4), e0175367. doi:10.1371/journal.pone.0175367 [PubMed: 28384302]

Gibson-Young L, Martinasek M, Tamulevicius N, Fortner M, & Alanazi AM (2020). Examining electronic nicotine delivery system use and perception of use among college students with and without asthma across the South. J Am Coll Health, 1–8. doi:10.1080/07448481.2020.1842414

Gu Q, Ni D, & Lee LY (2008). Expression of neuronal nicotinic acetylcholine receptors in rat vagal pulmonary sensory neurons. Respiratory physiology & neurobiology, 161(1), 87–91. doi:10.1016/j.resp.2007.11.006 [PubMed: 18206429]

Gu S, Matta JA, Lord B, Harrington AW, Sutton SW, Davini WB, & Bredt DS (2016). Brain alpha7 Nicotinic Acetylcholine Receptor Assembly Requires NACHO. Neuron, 89(5), 948–955. doi:10.1016/j.neuron.2016.01.018 [PubMed: 26875622]

Guedes AG, Deshpande DA, Dileepan M, Walseth TF, Panettieri RA Jr., Subramanian S, & Kannan MS (2015). CD38 and airway hyper-responsiveness: studies on human airway smooth muscle cells and mouse models. Canadian journal of physiology and pharmacology, 93(2), 145–153. doi:10.1139/cjpp-2014-0410 [PubMed: 25594684]

Hahn HL, Lang M, Bleicher S, Zwerenz S, & Rausch C (1992). Nicotine-induced airway smooth muscle contraction: neural mechanisms involving the airway epithelium. Functional and histologic studies in vitro. The Clinical investigator, 70(3-4), 252–262. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1355677 [PubMed: 1355677]

Hartiala JJ, Mapp C, Mitchell RA, & Gold WM (1985). Nicotine-induced respiratory effects of cigarette smoke in dogs. Journal of applied physiology, 59(1), 64–71. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2863247 [PubMed: 2863247]

He F, Li B, Zhao Z, Zhou Y, Hu G, Zou W, … Ran P (2014). The pro-proliferative effects of nicotine and its underlying mechanism on rat airway smooth muscle cells. PloS one, 9(4), e93508. doi:10.1371/journal.pone.0093508 [PubMed: 24690900]

Hirota N, & Martin JG (2013). Mechanisms of airway remodeling. Chest, 144(3), 1026–1032. doi:10.1378/chest.12-3073 [PubMed: 24008953]

Hong JL, & Lee LY (1996). Cigarette smoke-induced bronchoconstriction: causative agents and role of thromboxane receptors. Journal of applied physiology, 81(5), 2053–2059. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8941529 [PubMed: 8941529]

Hsieh FH (2016). Evidence vs advocacy in the e-cigarette debate: to vape or not to vape, that is the question. Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology, 116(2), 89–90. doi:10.1016/j.anai.2015.11.018

Jankowski M, Brozek G, Lawson J, Skoczynski S, & Zejda JE (2017). E-smoking: Emerging public health problem? Int J Occup Med Environ Health, 30(3), 329–344. doi:10.13075/ijomeh.1896.01046 [PubMed: 28481369]

Javed F, Kellesarian SV, Sundar IK, Romanos GE, & Rahman I (2017). Recent updates on electronic cigarette aerosol and inhaled nicotine effects on periodontal and pulmonary tissues. Oral Dis. doi:10.1111/odi.12652

Jia L, Delmotte P, Aravamudan B, Pabelick CM, Prakash YS, & Sieck GC (2013). Effects of the inflammatory cytokines TNF-alpha and IL-13 on stromal interaction molecule-1 aggregation in human airway smooth muscle intracellular Ca(2+) regulation. American journal of respiratory cell and molecular biology, 49(4), 601–608. doi:10.1165/rcmb.2013-0040OC [PubMed: 23713409]

Jiang Y, Dai A, Zhou Y, Peng G, Hu G, Li B, … Ran P (2014). Nicotine elevated intracellular Ca(2)(+) in rat airway smooth muscle cells via activating and up-regulating alpha7-nicotinic acetylcholine receptor. Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology, 33(2), 389–401. doi:10.1159/000356678

Kaur D, Gomez E, Doe C, Berair R, Woodman L, Saunders R, … Brightling CE (2015). IL-33 drives airway hyper-responsiveness through IL-13-mediated mast cell: airway smooth muscle crosstalk. Allergy, 70(5), 556–567. doi:10.1111/all.12593 [PubMed: 25683166]

Kaur G, Muthumalage T, & Rahman I (2018). Mechanisms of toxicity and biomarkers of flavoring and flavor enhancing chemicals in emerging tobacco and non-tobacco products. Toxicol Lett, 288, 143–155. doi:10.1016/j.toxlet.2018.02.025 [PubMed: 29481849]

Koopmans T, Anaparti V, Castro-Piedras I, Yarova P, Irechukwu N, Nelson C, … Wright DB (2014). Ca2+ handling and sensitivity in airway smooth muscle: emerging concepts for mechanistic understanding and therapeutic targeting. Pulmonary pharmacology & therapeutics, 29(2), 108–120. doi:10.1016/j.pupt.2014.05.001 [PubMed: 24831539]

Krasteva G, Canning BJ, Hartmann P, Veres TZ, Papadakis T, Muhlfeld C, … Kummer W (2011). Cholinergic chemosensory cells in the trachea regulate breathing. Proceedings of the National Academy of Sciences of the United States of America, 108(23), 9478–9483. doi:10.1073/pnas.1019418108 [PubMed: 21606356]

Lam DC, Luo SY, Fu KH, Lui MM, Chan KH, Wistuba II, … Minna JD (2016). Nicotinic acetylcholine receptor expression in human airway correlates with lung function. American journal of physiology. Lung cellular and molecular physiology, 310(3), L232–239. doi:10.1152/ajplung.00101.2015 [PubMed: 26608528]

Lam M, Lamanna E, & Bourke JE (2019). Regulation of Airway Smooth Muscle Contraction in Health and Disease. Adv Exp Med Biol, 1124, 381–422. doi:10.1007/978-981-13-5895-1_16 [PubMed: 31183836]

Lee LY, Burki NK, Gerhardstein DC, Gu Q, Kou YR, & Xu J (2007). Airway irritation and cough evoked by inhaled cigarette smoke: role of neuronal nicotinic acetylcholine receptors. Pulmonary pharmacology & therapeutics, 20(4), 355–364. doi:10.1016/j.pupt.2006.10.007 [PubMed: 17137814]

Lee LY, & Gu Q (2009). Cough sensors. IV. Nicotinic membrane receptors on cough sensors. Handbook of experimental pharmacology(187), 77–98. doi:10.1007/978-3-540-79842-2_5 [PubMed: 18825337]

Leonard S, & Bertrand D (2001). Neuronal nicotinic receptors: from structure to function. Nicotine & tobacco research : official journal of the Society for Research on Nicotine and Tobacco, 3(3), 203–223. doi:10.1080/14622200110050213 [PubMed: 11506765]

Lerner CA, Sundar IK, Yao H, Gerloff J, Ossip DJ, McIntosh S, … Rahman I (2015). Vapors produced by electronic cigarettes and e-juices with flavorings induce toxicity, oxidative stress, and inflammatory response in lung epithelial cells and in mouse lung. PloS one, 10(2), e0116732. doi:10.1371/journal.pone.0116732 [PubMed: 25658421]

Lin CC, Lee IT, Yang YL, Lee CW, Kou YR, & Yang CM (2010). Induction of COX-2/PGE(2)/IL-6 is crucial for cigarette smoke extract-induced airway inflammation: Role of TLR4-dependent NADPH oxidase activation. Free Radic Biol Med, 48(2), 240–254. doi:10.1016/j.freeradbiomed.2009.10.047 [PubMed: 19892012]

Lo D, Kennedy JL, Kurten RC, Panettieri RA Jr., & Koziol-White CJ (2018). Modulation of airway hyperresponsiveness by rhinovirus exposure. Respir Res, 19(1), 208. doi:10.1186/s12931-018-0914-9 [PubMed: 30373568]

Loxham M, Davies DE, & Blume C (2014). Epithelial function and dysfunction in asthma. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology, 44(11), 1299–1313. doi:10.1111/cea.12309 [PubMed: 24661647]

Lucchiari C, Masiero M, Veronesi G, Maisonneuve P, Spina S, Jemos C, … Pravettoni G (2016). Benefits of E-Cigarettes Among Heavy Smokers Undergoing a Lung Cancer Screening Program: Randomized Controlled Trial Protocol. JMIR research protocols, 5(1), e21. doi:10.2196/resprot.4805 [PubMed: 26842790]

Lundback B, Backman H, Lotvall J, & Ronmark E (2016). Is asthma prevalence still increasing? Expert review of respiratory medicine, 10(1), 39–51. doi:10.1586/17476348.2016.1114417 [PubMed: 26610152]

Manuyakorn W, Howarth PH, & Holgate ST (2013). Airway remodelling in asthma and novel therapy. Asian Pacific journal of allergy and immunology / launched by the Allergy and Immunology Society of Thailand, 31(1), 3–10. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/23517388

Matsumoto H, Hirata Y, Otsuka K, Iwata T, Inazumi A, Niimi A, … Mishima M (2012). Interleukin-13 enhanced Ca2+ oscillations in airway smooth muscle cells. Cytokine, 57(1), 19–24. doi:10.1016/j.cyto.2011.10.014 [PubMed: 22078634]

Matta JA, Gu S, Davini WB, Lord B, Siuda ER, Harrington AW, & Bredt DS (2017). NACHO Mediates Nicotinic Acetylcholine Receptor Function throughout the Brain. Cell Rep, 19(4), 688–696. doi:10.1016/j.celrep.2017.04.008 [PubMed: 28445721]

May SM, & Li JT (2015). Burden of chronic obstructive pulmonary disease: healthcare costs and beyond. Allergy and asthma proceedings : the official journal of regional and state allergy societies, 36(1), 4–10. doi:10.2500/aap.2015.36.3812

McEvoy CT, & Spindel ER (2017). Pulmonary Effects of Maternal Smoking on the Fetus and Child: Effects on Lung Development, Respiratory Morbidities, and Life Long Lung Health. Paediatr Respir Rev, 21, 27–33. doi:10.1016/j.prrv.2016.08.005 [PubMed: 27639458]

Meo SA, & Al Asiri SA (2014). Effects of electronic cigarette smoking on human health. European review for medical and pharmacological sciences, 18(21), 3315–3319. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/25487945 [PubMed: 25487945]

Millar NS (2008). RIC-3: a nicotinic acetylcholine receptor chaperone. Br J Pharmacol, 153 Suppl 1, S177–183. doi:10.1038/sj.bjp.0707661 [PubMed: 18246096]

Narumoto O, Horiguchi K, Horiguchi S, Moriwaki Y, Takano-Ohmuro H, Shoji S, … Yamashita N (2010). Down-regulation of secreted lymphocyte antigen-6/urokinase-type plasminogen activator receptor-related peptide-1 (SLURP-1), an endogenous allosteric alpha7 nicotinic acetylcholine receptor modulator, in murine and human asthmatic conditions. Biochem Biophys Res Commun, 398(4), 713–718. doi:10.1016/j.bbrc.2010.07.006 [PubMed: 20621062]

Narumoto O, Niikura Y, Ishii S, Morihara H, Okashiro S, Nakahari T, … Yamashita N (2013). Effect of secreted lymphocyte antigen-6/urokinase-type plasminogen activator receptor-related peptide-1

(SLURP-1) on airway epithelial cells. Biochem Biophys Res Commun, 438(1), 175–179. doi:10.1016/j.bbrc.2013.07.048 [PubMed: 23876317]

Noble PB, Pascoe CD, Lan B, Ito S, Kistemaker LE, Tatler AL, … West AR (2014). Airway smooth muscle in asthma: linking contraction and mechanotransduction to disease pathogenesis and remodelling. Pulmonary pharmacology & therapeutics, 29(2), 96–107. doi:10.1016/j.pupt.2014.07.005 [PubMed: 25062835]

Oh AY, & Kacker A (2014). Do electronic cigarettes impart a lower potential disease burden than conventional tobacco cigarettes? Review on E-cigarette vapor versus tobacco smoke. The Laryngoscope, 124(12), 2702–2706. doi:10.1002/lary.24750 [PubMed: 25302452]

Orellana-Barrios MA, Payne D, Mulkey Z, & Nugent K (2015). Electronic Cigarettes-A Narrative Review for Clinicians. The American journal of medicine, 128(7), 674–681. doi:10.1016/j.amjmed.2015.01.033 [PubMed: 25731134]

Pain M, Bermudez O, Lacoste P, Royer PJ, Botturi K, Tissot A, … Magnan A (2014). Tissue remodelling in chronic bronchial diseases: from the epithelial to mesenchymal phenotype. European respiratory review : an official journal of the European Respiratory Society, 23(131), 118–130. doi:10.1183/09059180.00004413 [PubMed: 24591669]

Parulekar AD, Atik MA, & Hanania NA (2014). Periostin, a novel biomarker of TH2-driven asthma. Current opinion in pulmonary medicine, 20(1), 60–65. doi:10.1097/MCP.0000000000000005 [PubMed: 24247042]

Pieper MP (2012). The non-neuronal cholinergic system as novel drug target in the airways. Life Sci, 91(21-22), 1113–1118. doi:10.1016/j.lfs.2012.08.030 [PubMed: 22982180]

Postma DS, Bush A, & van den Berge M (2015). Risk factors and early origins of chronic obstructive pulmonary disease. Lancet, 385(9971), 899–909. doi:10.1016/S0140-6736(14)60446-3 [PubMed: 25123778]

Prakash YS (2013). Airway smooth muscle in airway reactivity and remodeling: what have we learned? American journal of physiology. Lung cellular and molecular physiology, 305(12), L912–933. doi:10.1152/ajplung.00259.2013 [PubMed: 24142517]

Prakash YS (2016). Emerging concepts in smooth muscle contributions to airway structure and function: implications for health and disease. American journal of physiology. Lung cellular and molecular physiology, 311(6), L1113–L1140. doi:10.1152/ajplung.00370.2016 [PubMed: 27742732]

Racke K, & Matthiesen S (2004). The airway cholinergic system: physiology and pharmacology. Pulmonary pharmacology & therapeutics, 17(4), 181–198. doi:10.1016/j.pupt.2004.03.001 [PubMed: 15219263]

Rex EB, Shukla N, Gu S, Bredt D, & DiSepio D (2017). A Genome-Wide Arrayed cDNA Screen to Identify Functional Modulators of alpha7 Nicotinic Acetylcholine Receptors. SLAS Discov, 22(2), 155–165. doi:10.1177/1087057116676086 [PubMed: 27789755]

Richardson PS, & Peatfield AC (1987). The control of airway mucus secretion. European journal of respiratory diseases. Supplement, 153, 43–51. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/3322868 [PubMed: 3322868]

Rincon M, & Irvin CG (2012). Role of IL-6 in asthma and other inflammatory pulmonary diseases. Int J Biol Sci, 8(9), 1281–1290. doi:10.7150/ijbs.4874 [PubMed: 23136556]

Robinson MB, Deshpande DA, Chou J, Cui W, Smith S, Langefeld C, … Hawkins GA (2015). IL-6 trans-signaling increases expression of airways disease genes in airway smooth muscle. American journal of physiology. Lung cellular and molecular physiology, 309(2), L129–138. doi:10.1152/ajplung.00288.2014 [PubMed: 26001777]

Rogers DF (2002). Pharmacological regulation of the neuronal control of airway mucus secretion. Curr Opin Pharmacol, 2(3), 249–255. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/12020465 [PubMed: 12020465]

Rowell TR, & Tarran R (2015). Will chronic e-cigarette use cause lung disease? American journal of physiology. Lung cellular and molecular physiology, 309(12), L1398–1409. doi:10.1152/ajplung.00272.2015 [PubMed: 26408554]

Sathish V, Freeman MR, Long E, Thompson MA, Pabelick CM, & Prakash YS (2015). Cigarette Smoke and Estrogen Signaling in Human Airway Smooth Muscle. Cellular physiology and

biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology, 36(3), 1101–1115. doi:10.1159/000430282

Sathish V, Thompson MA, Sinha S, Sieck GC, Prakash YS, & Pabelick CM (2014). Inflammation, caveolae and CD38-mediated calcium regulation in human airway smooth muscle. Biochimica et biophysica acta, 1843(2), 346–351. doi:10.1016/j.bbamcr.2013.11.011 [PubMed: 24275509]

Sathish V, Xu A, Karmazyn M, Sims SM, & Narayanan N (2006). Mechanistic basis of differences in Ca2+ -handling properties of sarcoplasmic reticulum in right and left ventricles of normal rat myocardium. Am J Physiol Heart Circ Physiol, 291(1), H88–96. doi:10.1152/ajpheart.01372.2005 [PubMed: 16461368]

Schneider NG, Lunell E, Olmstead RE, & Fagerstrom KO (1996). Clinical pharmacokinetics of nasal nicotine delivery. A review and comparison to other nicotine systems. Clinical pharmacokinetics, 31(1), 65–80. doi:10.2165/00003088-199631010-00005 [PubMed: 8827400]

Schneider NG, Olmstead RE, Franzon MA, & Lunell E (2001). The nicotine inhaler: clinical pharmacokinetics and comparison with other nicotine treatments. Clinical pharmacokinetics, 40(9), 661–684. doi:10.2165/00003088-200140090-00003 [PubMed: 11605715]

Sheehan WJ, & Phipatanakul W (2015). Difficult-to-control asthma: epidemiology and its link with environmental factors. Current opinion in allergy and clinical immunology, 15(5), 397–401. doi:10.1097/ACI.0000000000000195 [PubMed: 26226354]

Sitapara RA, Antoine DJ, Sharma L, Patel VS, Ashby CR Jr., Gorasiya S, … Mantell LL (2014). The alpha7 nicotinic acetylcholine receptor agonist GTS-21 improves bacterial clearance in mice by restoring hyperoxia-compromised macrophage function. Mol Med, 20, 238–247. doi:10.2119/molmed.2013.00086 [PubMed: 24664237]

St Claire S, Gouda H, Schotte K, Fayokun R, Fu D, Varghese C, & Prasad VM (2020). Lung health, tobacco, and related products: gaps, challenges, new threats, and suggested research. American journal of physiology. Lung cellular and molecular physiology, 318(5), L1004–L1007. doi:10.1152/ajplung.00101.2020 [PubMed: 32233791]

Su X, Matthay MA, & Malik AB (2010). Requisite role of the cholinergic alpha7 nicotinic acetylcholine receptor pathway in suppressing Gram-negative sepsis-induced acute lung inflammatory injury. J Immunol, 184(1), 401–410. doi:10.4049/jimmunol.0901808 [PubMed: 19949071]

Svenningsen S, & Nair P (2017). Asthma Endotypes and an Overview of Targeted Therapy for Asthma. Front Med (Lausanne), 4, 158. doi:10.3389/fmed.2017.00158 [PubMed: 29018800]

Townsend EA, Sathish V, Thompson MA, Pabelick CM, & Prakash YS (2012). Estrogen effects on human airway smooth muscle involve cAMP and protein kinase A. Am J Physiol Lung Cell Mol Physiol, 303(10), L923–928. doi:10.1152/ajplung.00023.2012 [PubMed: 23002077]

Traboulsi H, Cherian M, Abou Rjeili M, Preteroti M, Bourbeau J, Smith BM, … Baglole CJ (2020). Inhalation Toxicology of Vaping Products and Implications for Pulmonary Health. Int J Mol Sci, 21(10). doi:10.3390/ijms21103495

van der Velden VH, & Hulsmann AR (1999). Autonomic innervation of human airways: structure, function, and pathophysiology in asthma. Neuroimmunomodulation, 6(3), 145–159. doi:26376 [PubMed: 10213912]

Viegi G, Maio S, Pistelli F, Baldacci S, & Carrozzi L (2006). Epidemiology of chronic obstructive pulmonary disease: health effects of air pollution. Respirology, 11(5), 523–532. doi:10.1111/j.1440-1843.2006.00886.X [PubMed: 16916323]

Vogel ER, VanOosten SK, Holman MA, Hohbein DD, Thompson MA, Vassallo R,… Pabelick CM (2014). Cigarette smoke enhances proliferation and extracellular matrix deposition by human fetal airway smooth muscle. American journal of physiology. Lung cellular and molecular physiology, 307(12), L978–986. doi:10.1152/ajplung.00111.2014 [PubMed: 25344066]

Walton KM, Abrams DB, Bailey WC, Clark D, Connolly GN, Djordjevic MV, … Hatsukami DK (2015). NIH electronic cigarette workshop: developing a research agenda. Nicotine & tobacco research : official journal of the Society for Research on Nicotine and Tobacco, 17(2), 259–269. doi:10.1093/ntr/ntu214 [PubMed: 25335949]

Wang H, Yu M, Ochani M, Amella CA, Tanovic M, Susarla S, … Tracey KJ (2003). Nicotinic acetylcholine receptor alpha7 subunit is an essential regulator of inflammation. Nature, 421(6921), 384–388. doi:10.1038/nature01339 [PubMed: 12508119]

Wang Y, Pereira EF, Maus AD, Ostlie NS, Navaneetham D, Lei S, … Conti-Fine BM (2001). Human bronchial epithelial and endothelial cells express alpha7 nicotinic acetylcholine receptors. Molecular pharmacology, 60(6), 1201–1209. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11723227 [PubMed: 11723227]

Wright DB, Tripathi S, Sikarwar A, Santosh KT, Perez-Zoghbi J, Ojo OO, … Schaafsma D (2013). Regulation of GPCR-mediated smooth muscle contraction: implications for asthma and pulmonary hypertension. Pulmonary pharmacology & therapeutics, 26(1), 121–131. doi:10.1016/j.pupt.2012.06.005 [PubMed: 22750270]

Wurst KE, Kelly-Reif K, Bushnell GA, Pascoe S, & Barnes N (2016). Understanding asthma-chronic obstructive pulmonary disease overlap syndrome. Respiratory medicine, 110, 1–11. doi:10.1016/j.rmed.2015.10.004 [PubMed: 26525374]

Wylam ME, Sathish V, VanOosten SK, Freeman M, Burkholder D, Thompson MA, … Prakash YS (2015). Mechanisms of Cigarette Smoke Effects on Human Airway Smooth Muscle. PloS one, 10(6), e0128778. doi:10.1371/journal.pone.0128778 [PubMed: 26075746]

Zoli M, Pistillo F, & Gotti C (2015). Diversity of native nicotinic receptor subtypes in mammalian brain. Neuropharmacology, 96(Pt B), 302–311. doi:10.1016/j.neuropharm.2014.11.003 [PubMed: 25460185]

Zuo H, Han B, Poppinga WJ, Ringnalda L, Kistemaker LEM, Halayko AJ, … Schmidt M (2018). Cigarette smoke up-regulates PDE3 and PDE4 to decrease cAMP in airway cells. Br J Pharmacol, 175(14), 2988–3006. doi:10.1111/bph.14347 [PubMed: 29722436]

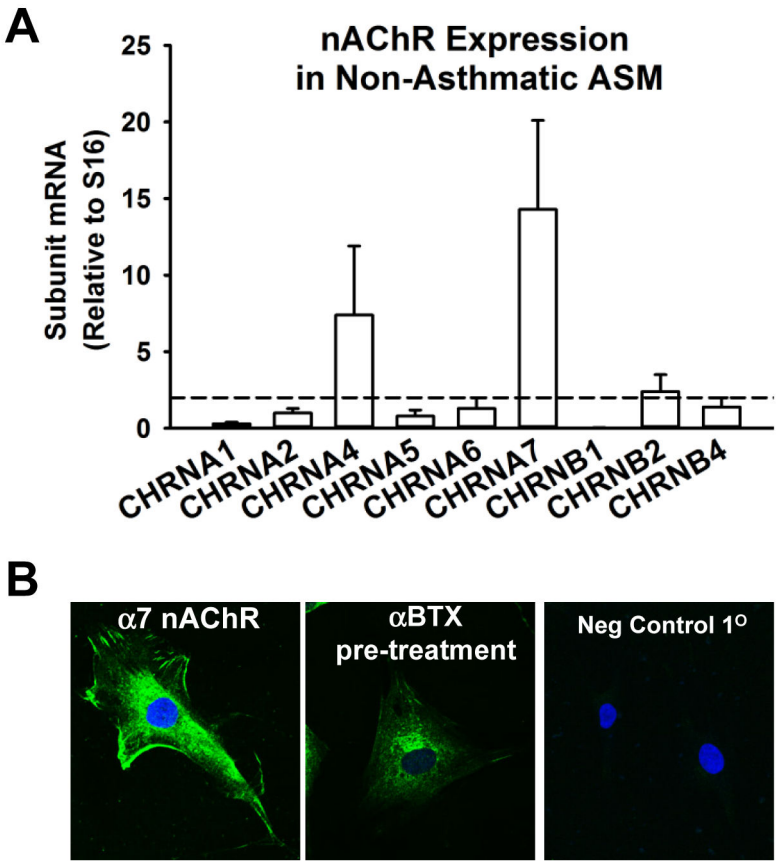

Figure 1:

Expression of nicotinic cholinergic receptor (nAChR) subunits in human airway smooth muscle (ASM) cells. mRNA studies in non-asthmatic human ASM cells show significantly higher expression of the α4 and particularly α7 subunits with small expression of β2 subunit, but otherwise minimal to no expression of other subunits (A). α7nAChR is expressed on ASM cell plasma membrane, evidenced by immunofluorescence (green) that is prevented by alpha-bungarotoxin (αBTX) binding of the receptor (B). Data represented as mean ± SEM (N=4).

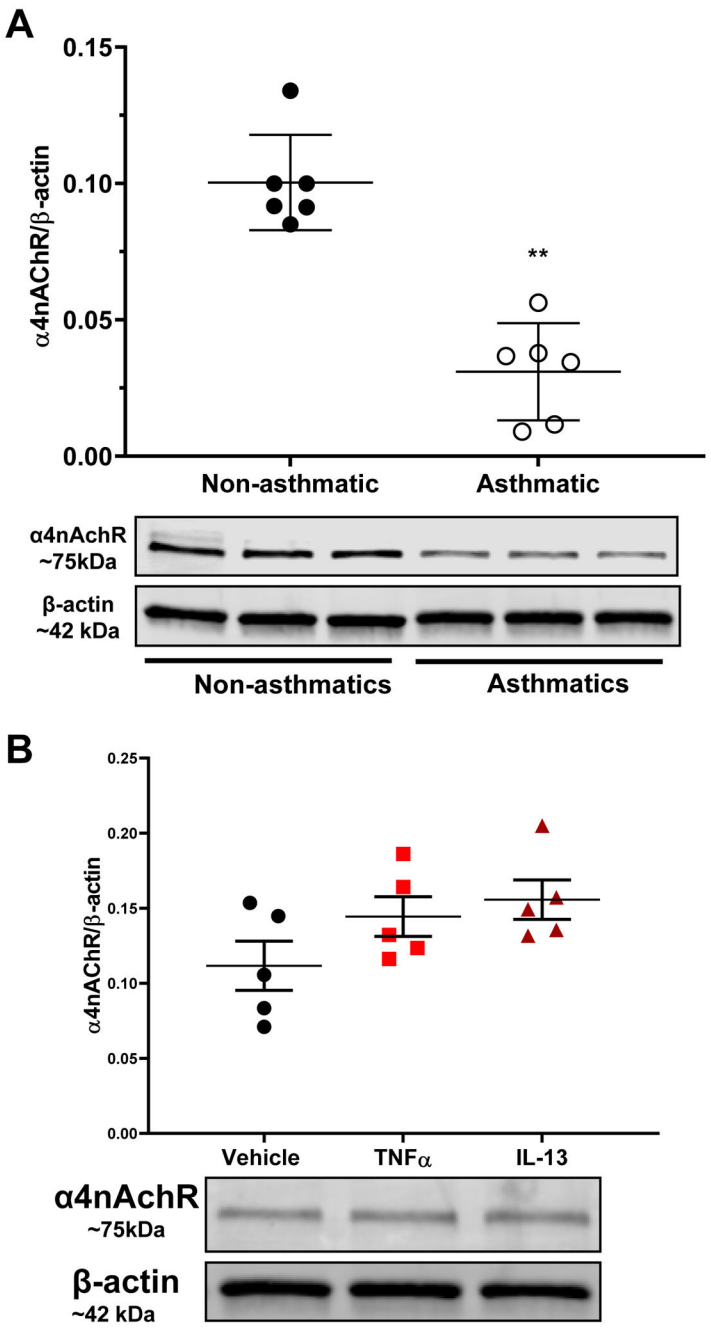

Figure 2:

α4nAChR in human ASM cells. Western blotting showed that expression of α4nAChR is lower in asthmatic ASM compared to non-asthmatics (A). Exposure of non-asthmatic ASM cells to pro-inflammatory cytokines (20ng/ml TNFα or 50ng/ml IL-13) resulted in only slight increases in α4nAChR compared to vehicle (B). ** $p<0.05$ vs. non-asthmatics. Data represented as mean ± SEM (N=5-6).

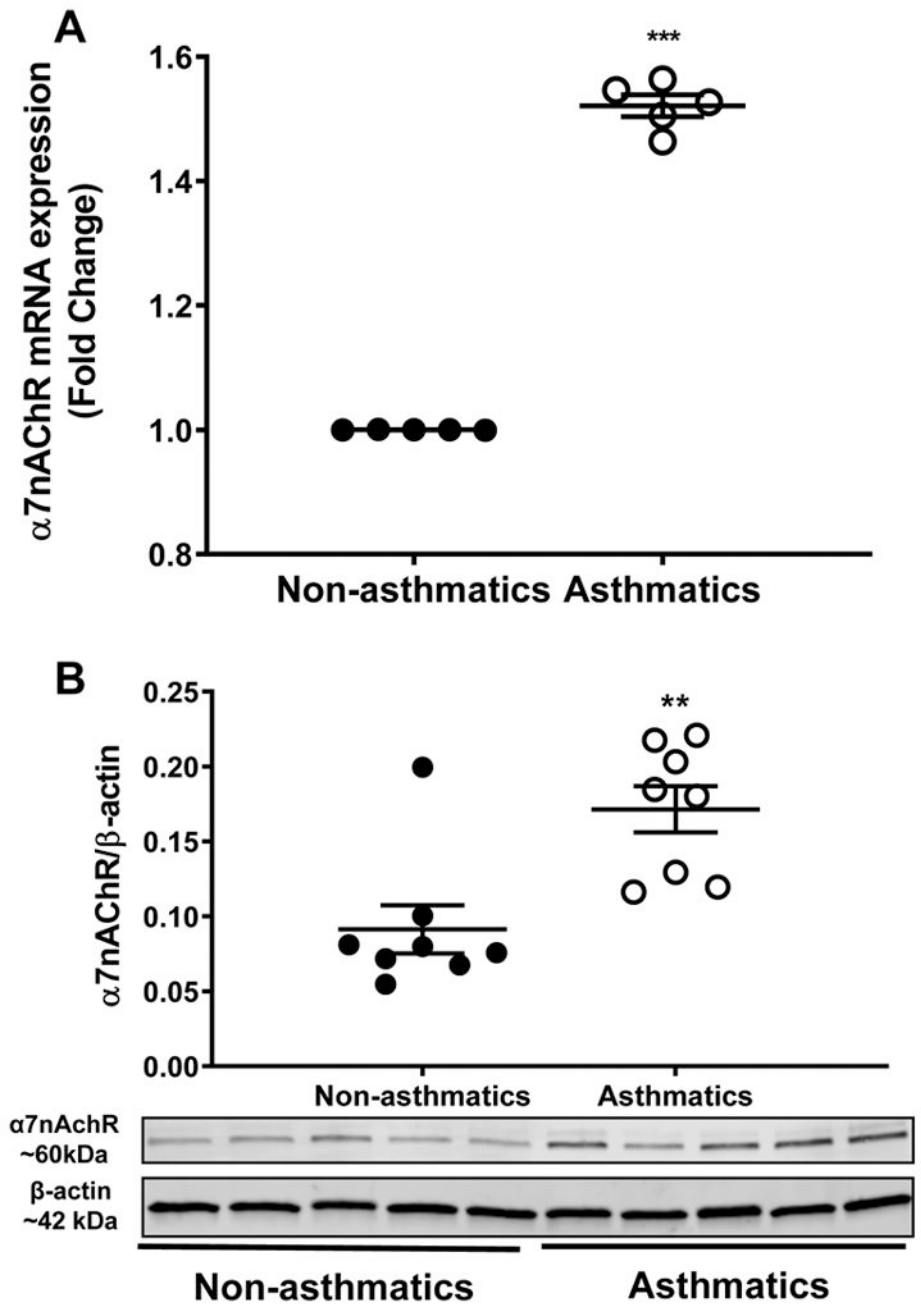

Figure 3:

α7nAChR in human ASM cells. qRT-PCR (A) and western blotting (B) of ASM from non-asthmatics vs. asthmatics showed higher expression of α7nAChR in asthmatics. ** $p<0.05$, *** $p<0.001$ vs. non-asthmatics. Data represented as mean ± SEM (N=5-8).

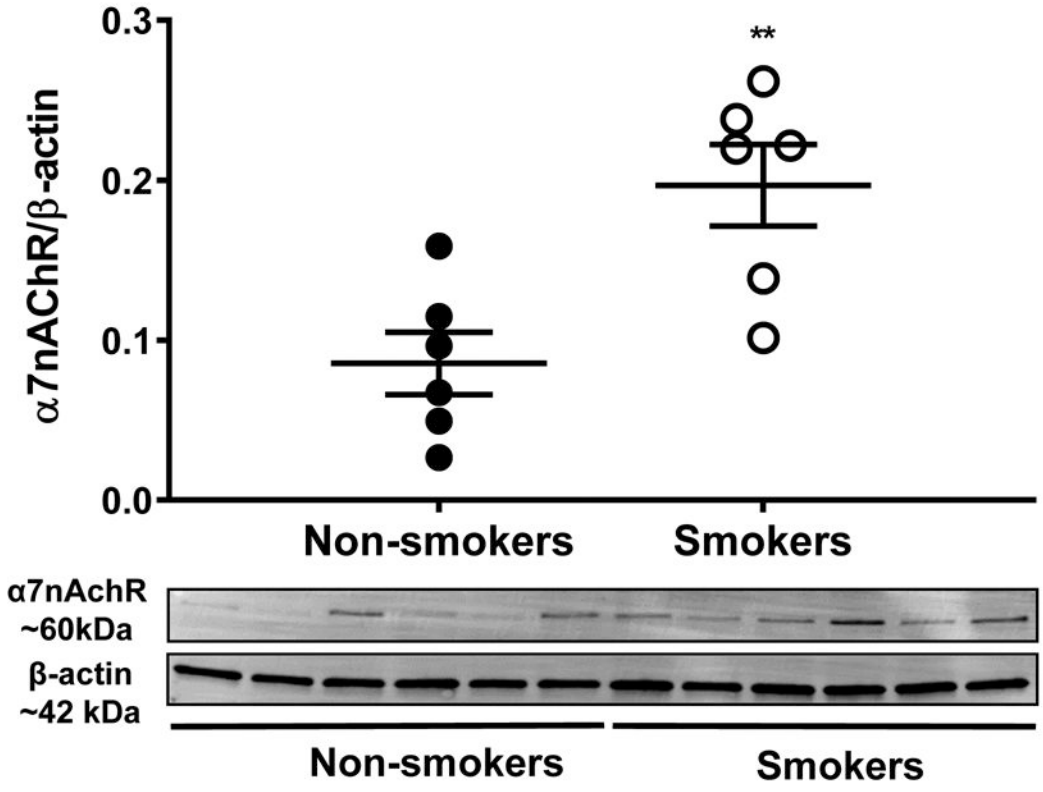

Figure 4:

α7nAChR in ASM of smokers. Western analysis showed higher expression of α7nAChR in human ASM cells of smokers compared to non-smokers. ** $p<0.01$ vs. Non-smokers. Data represented as mean ± SEM (N=6).

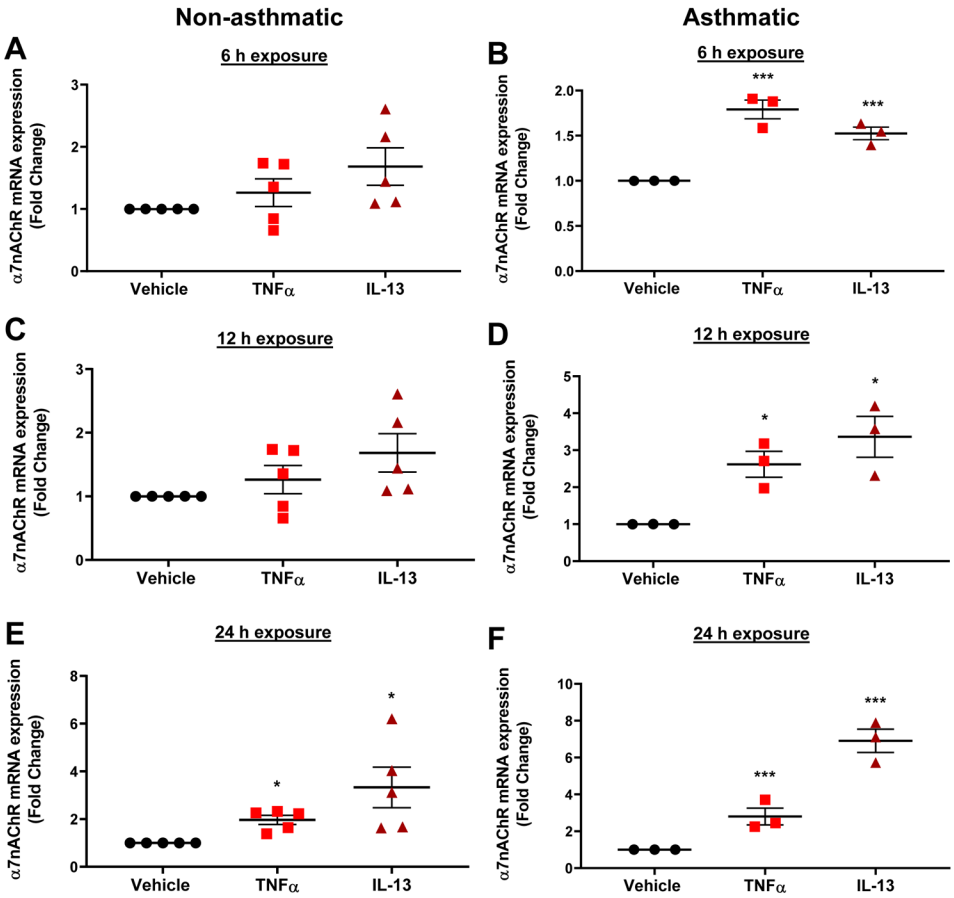

Figure 5:

Effect of inflammatory cytokines on α7nAChR in human ASM cells. qRT-PCR analysis showed that in non-asthmatic ASM, 24h of TNFα or IL-13 exposure significantly increased α7nAChR expression (E) while 6 h or 12 h only trended towards an increase for either cytokine (A and C). On the other hand, expression of α7nAChR was significantly increased for all time points for asthmatic ASM cells (B, D, F). *p<0.05, ***p<0.001 vs. vehicle. Data represented as mean ± SEM (N=3-5).

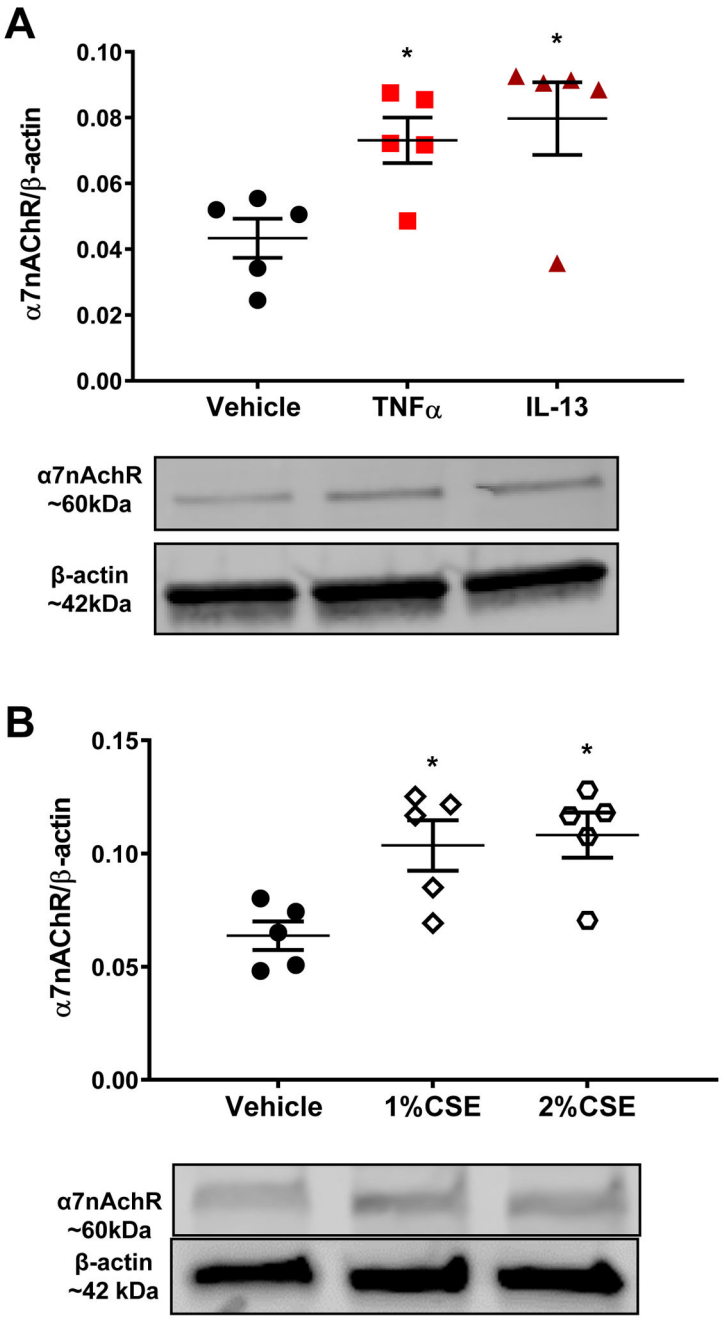

Figure 6:

Effect of inflammatory cytokines and cigarette smoke on α7nAChR in human ASM. The effect of pro-inflammatory cytokines (TNFα and IL-13) and cigarette smoke extract (CSE; 1% and 2%) on α7nAChR was explored by western blotting. All these insults resulted in significant increase in α7nAChR expression. *p<0.05 vs. vehicle. Data represented as mean ± SEM (N=5).

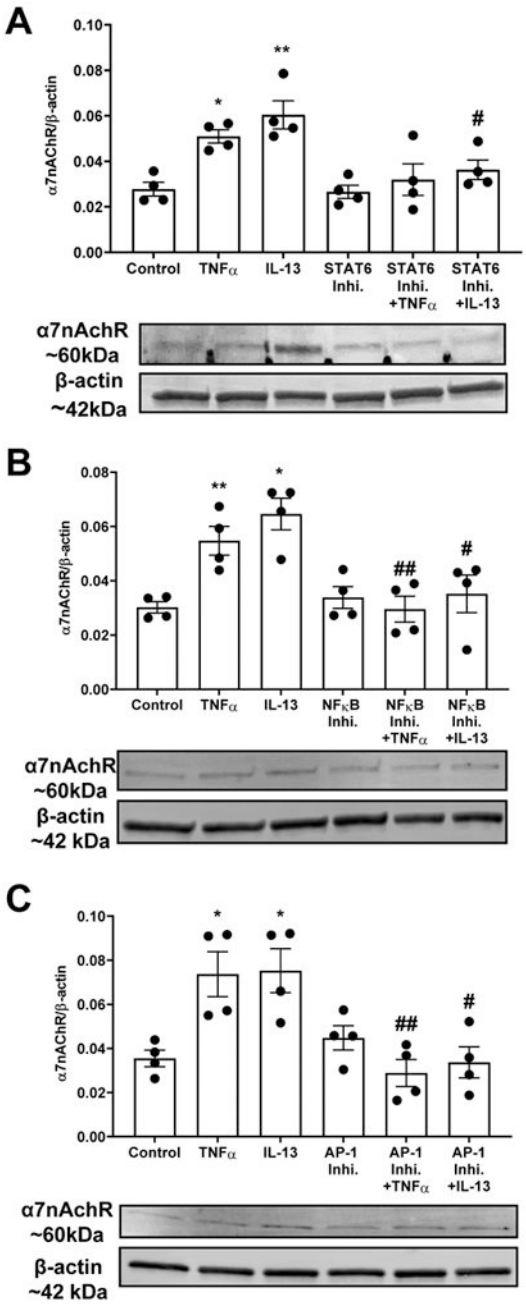

Figure 7:

Inflammation-associated nuclear signals regulate α7nAChR in human ASM. In non-asthmatic human ASM cells, pharmacological inhibition of STAT6 (20 nM AS151749; A), NFκB (20 μM SN50; B) or AP-1 inhibitor (1 μM SR1302; C) significantly blunted the enhancing effects of TNFα or IL-13 on α7nAChR, with STAT6 inhibition showing specificity for IL-13. *p<0.05, **p<0.01 vs. vehicle, #p<0.05, ##p<0.01 vs. respective inflammatory cytokine. Data represented as mean ± SEM (N=4).

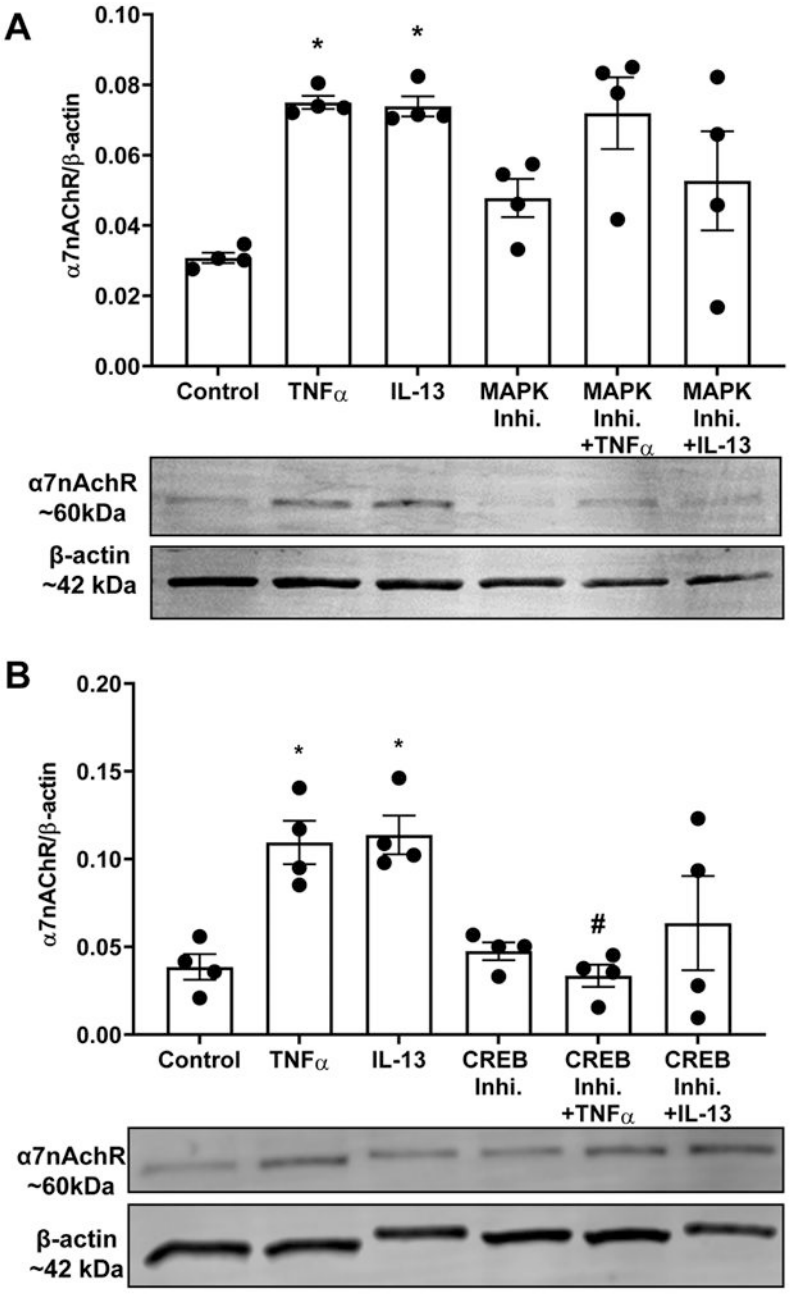

Figure 8:

Inflammation-associated cytosolic signals regulate α7nAChR in human ASM. In non-asthmatic human ASM cells, pharmacological inhibition of p42/44 MAPK (2 μM PD98059; A) or CREB (5 μM 666-15; B) significantly blunted the enhancing effects of TNFα or IL-13 on α7nAChR. CREB inhibition showed specificity for TNFα. *p<0.05 vs. Vehicle, #p<0.05 vs. respective inflammatory cytokine. Data represented as mean ± SEM (N=4).

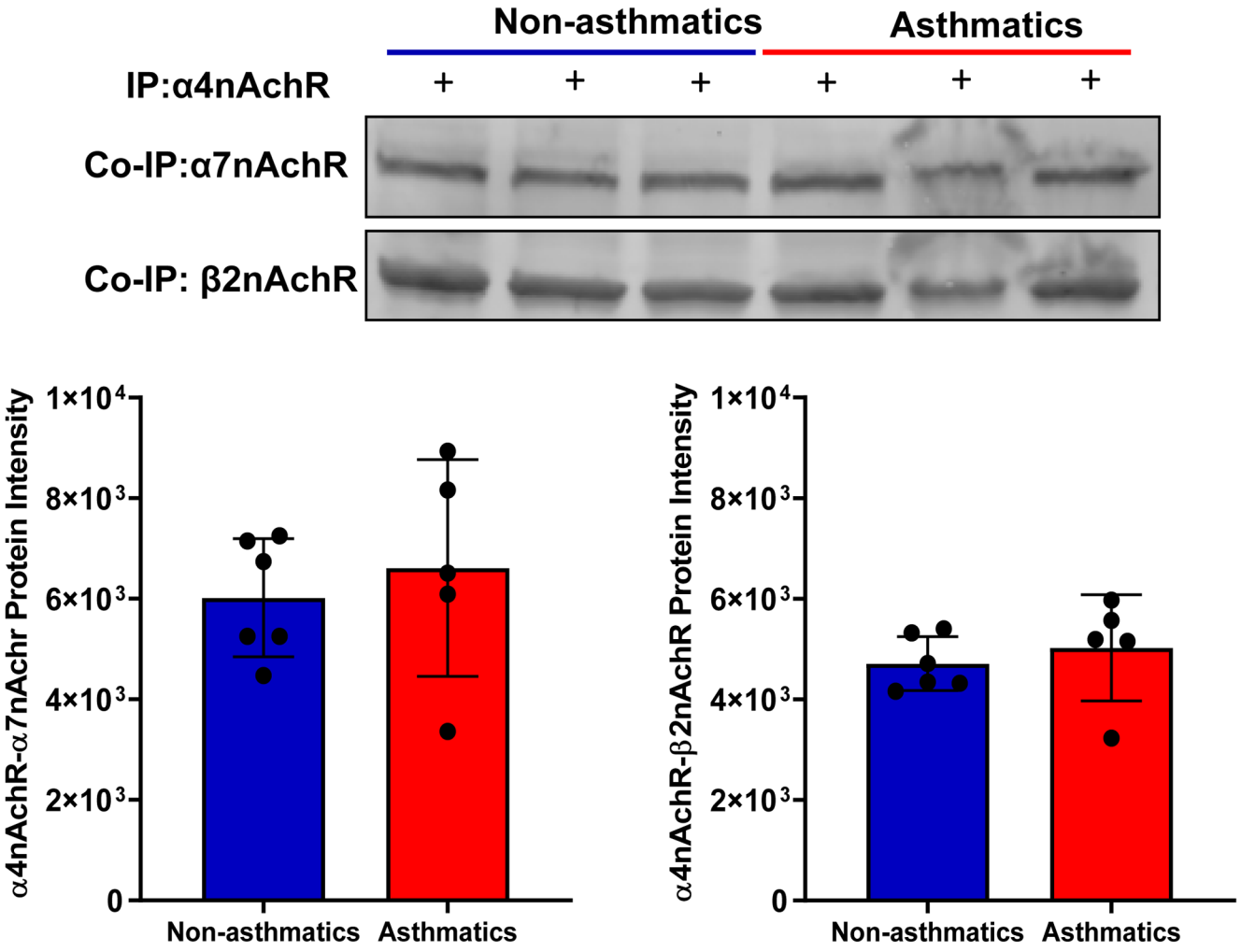

Figure 9:

α4nAChR interacts with α7nAChR and β2nAChR in human ASM. Immunoprecipitation with α4nAChR and subsequent co-immunoprecipitation of α7nAChR and β2nAChR using western blot analyses revealed that α7nAChR and β2nAChR interacts with α4nAChR in both non-asthmatic and asthmatic ASM cells with no significant difference observed between the groups. Data represented as mean ± SEM (N=5-6).