Involvement of alpha-7 nicotinic receptor (α7nAChR) in respiratory syncytial virus infection-induced asthma in rats

Jun Wang ( wangjunps123@163.com )
Nanchang University

Zifeng Wei
Jiangxi Provincial People's Hospital

Xu Zhong
Medical College of Nanchang University

Lixue Dai
Medical College of Nanchang University

Research

Keywords: Asthma, alpha-7 nicotinic receptor, nerve growth factor, respiratory syncytial virus

DOI: https://doi.org/10.21203/rs.3.rs-320221/v1

License: ☑️ This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License
Abstract

This study aimed to investigate the role of α-7 nicotinic receptor (α7nAChR) in rats with asthma caused by respiratory syncytial virus (RSV) infection. An asthmatic rat model was established by RSV infection and confirmed based on pathological changes. sh-α7nAChR lentivirus was constructed to reduce α7nAChR expression in rats. The animals were divided into a control group, a model group, a sh-α7nAChR lentivirus group, a nerve growth factor (NGF) antibody group, a dexamethasone (DXMS) group, a sh-α7nAChR lentivirus + NGF antibody group, and a sh-α7nAChR lentivirus + DXMS group. The expression of α7nAChR was detected by quantitative real-time PCR and western blotting. Serum levels of adrenaline and norepinephrine were detected by ELISA. Transmission electron microscopy determined the morphological change of chromaffin cells and synaptic vesicles. Immunofluorescence was used to detect the expression of synaptophysin (SYN) and nuclear factor-kappa B (NF-κB). Compared with the control group, the model group showed significantly increased expression of α7nAChR, SYN, and NF-κB. Compared with the model group, the sh-α7nAChR lentivirus, NGF antibody, DXMS, sh-α7nAChR lentivirus + NGF antibody, and sh-α7nAChR lentivirus + DXMS groups showed significantly decreased expression of α7nAChR (P < 0.05). After repeated infection with RSV, the number of chromaffin cells and synaptic vesicles increased, which were significantly reduced after silencing α7nAChR by NGF antibody or DXMS treatment. The model group showed significantly lower serum adrenaline than the control group (P < 0.05). Similarly, the serum adrenaline in the sh-α7nAChR lentivirus, sh-α7nAChR lentivirus + NGF antibody, and sh-α7nAChR lentivirus + DXMS groups, but not the NGF antibody and DXMS groups, was significantly lower than in the model group. Together, upregulation of α7nAChR is involved in RSV infection-induced asthma in rats. Silencing of α7nAChR reduces the numbers of chromaffin granules and synaptic vesicles and adrenaline release.

Introduction

Bronchial asthma is a common and frequently occurring respiratory disease, with paroxysmal wheezing, shortness of breath, chest tightness, and cough being the main symptoms. Asthma is characterized by chronic airway inflammatory reaction, which is associated with airway hyperresponsiveness (1). Infants with recurrent respiratory syncytial virus (RSV) infection have a high risk of bronchial asthma in adolescence (2, 3), but the pathogenesis is not yet fully known. Presently, bronchial asthma is treated using glucocorticoid and β-2-adrenergic receptor agonists (4). As a representative β-2-adrenergic receptor agonist, adrenaline is still the most effective treatment (5) and acts by binding with adrenergic receptors in the airway smooth muscle (5). Insufficient adrenaline secretion in the asthmatic state leads to unbinding of adrenaline with the receptors, which inhibits alleviation of bronchospasm during an asthmatic attack (6, 7).

Adrenaline is synthesized by adrenomedullary chromaffin cells and released into the circulatory system; the chromaffin cells originate from the neural crest and possess the phenotype and function of endocrine cells (8). Their differentiation is regulated by nerve growth factor (NGF) and glucocorticoids (9, 10). After continuous injection of NGF into pregnant rats, chromaffin cells from the offspring rats can transform
into sympathetic neurons (11). Similarly, primary cultured chromaffin cells gradually lose their endocrine phenotypes and are transformed into sympathetic neurons after NGF treatment; meanwhile, glucocorticoids could also block NGF-induced phenotypic transformation of chromaffin cells (12).

Seventeen subunits of nicotinic acetylcholine receptors (nAChR) have been identified based on molecular cloning technique: 10 α-subunits; four β-subunits; and one each of γ, δ, and epsilon subunits. These subunits assist the nACh receptor subtypes to regulate different physiological functions (13). Redistribution of nAChR subunits, primarily the α7nAChR, occurs in the adrenal medulla of asthmatic mice (14, 15). A previous study reported that α7nAChR was a potential therapeutic target for the treatment of type-2 innate lymphoid cells (ILC2s)-dependent asthma (16). However, to our knowledge, the function of α7nAChR in RSV infection-induced asthma has not yet been verified. In this study, we aimed to clarify the function of α7nAChR in RSV infection-induced asthma by silencing α7nAChR or by the application of NGF antibody or dexamethasone (DXMS).

**Materials And Methods**

*Construction of α7nAChR shRNA lentivirus*

Three pairs of interference sequences were designed and synthesized as per gene sequences. The conjugated products were transformed into susceptible bacteria and cultured overnight in a 24-well plate. Complete colonies were randomly selected from the plate. The positive clones were detected by PCR, sequenced, and validated. The bacteria with correct sequence of extraction and plasmid detection were amplified. The interfering lentiviruses were extracted by Axygen Plasmid Extraction Kit (Cat. APGX250, Axygen). Plasmid vector was detected by optical density (OD) values (data not shown). The sequences of shRNA for α7nAChR are listed in Table 1.

Briefly, 293T cells were digested with 0.05–0.25% trypsin and suspended as single cells in complete Dulbecco's Modified Eagle Medium (DMEM) (Hyclone/Thermo SH30023.01B). The cells were counted and inoculated into the dish (106 cells per 10-cm dish) and cultured overnight at 37°C in a 5% CO₂ incubator. Before transfection, the medium was removed and replaced with 5 mL Opti-MEM medium (31985, GIBCO). 9 µg Packaging Mix and 3 µg lentiviral expression plasmids were added into 1.5 mL Opti-MEM (preheated at 37°C). 36 µL lipofectamine 2000 was added to 1.5 mL Opti-MEM and kept at room temperature for 5 min. Lipofectamine 2000 solution, kept at room temperature for 20 min, was added to the dish, gently mixed, and incubated at 37°C in a 5% CO₂ incubator for 6 h. Then, the medium was replaced with complete medium (DMEM+10% fetal bovine serum [FBS]). After 48 h, the virus was collected and the titer tested.

*Animal model*

Experimental protocols were approved and supervised by the Animal Care and Use Committee of People's Hospital of Nanchang University (No. 2019093). In all, 42 male Sprague–Dawley rats (weight: 180±5 g) were purchased from Vital River Laboratories Co., Ltd. (Beijing, China) and maintained in specific
pathogen-free conditions at a temperature of 23±2°C, relative humidity of 45–65%, and a controlled 12/12 h light/dark cycle. The asthmatic rat model was established by repeated RSV infection as previously described (17). 12 weeks after infection, the rats were anesthetized by inhalation of isoflurane (5%) and decapitated. The health and behaviors were monitored every day and there was no death of the animals during the experiments.

RSV virus was obtained from the Institute of Microbiology, Xiangya Medical College, and rats were infected by RSV inhalation once a day (105 PFU/infection) for one week. Goat anti-rabbit NGF antibody (11050-MM06, Sino Biological) was injected intraperitoneally every week before the virus infection, and the negative control group was injected with virus-free medium. The source of infection was immediately isolated, and cages were replaced every three days and disinfected. The rats were sacrificed 12 weeks after infection. The animals were divided into seven groups (n=6 in each group): a control group; a model group (RSV-infected rats, 105 PFU/infected for one week); a sh-α7nAChR lentivirus group (200 µl, tail vein injection); a NGF antibody treatment group (anti-NGF, goat anti-rabbit β-NGF antibody was injected intraperitoneally every week before virus infection at a dose of 4 mL/kg/day for one week before infection); a dexamethasone (DXMS) treatment group (i.p. 2 mg/kg/day for one week before infection); a sh-α7nAChR lentivirus+NGF antibody group, and a sh-α7nAChR lentivirus+DXMS group. After 12 weeks’ treatment, the rats were anesthetized by isoflurane and decapitated. Pathological changes were observed in modeled rats by hematoxylin-eosin (HE) staining. Adrenal tissues were collected for subsequent experiments.

**Transmission electron microscopy**

The ultrastructure of rat chromaffin cells was detected by transmission electron microscopy. The dust and impurities on the surface of the samples were washed repeatedly with phosphate buffer, fixed for 2 h with 3% glutaraldehyde at room temperature. The tissues were washed three times with phosphate buffer for 10 min each time; dehydrated with 50%, 70%, 80%, and 90% ethanol for 15 min; and removed with 100% ethanol. The tissues were sectioned into 70-nm slices, which were then stained by 3% uranyl acetate and lead citrate and imaged with transmission electron microscopy (80 kV) (JEOL JEM-1230).

**Immunofluorescence**

The tissues were fixed with 4% paraformaldehyde for 15 min at room temperature, washed with PBS, and permeated with 0.5% Triton X-100 (PBS) at room temperature for 20 min. 5% BSA was used to block the unspecific staining (30 min at 37°C). The tissues were incubated with the antibodies against synaptophysin (1:200; ab32127, Abcam) and NF-κB (1:200; ab32536, Abcam) overnight at 4°C. After washing, Alexa Fluor 593 goat anti-mouse IgG (1:100; catalog no. CW0159S, CW BiotechCWBio, Beijing, China) was incubated with the slides for 30 min at room temperature. The images were taken using fluorescent microscopy. At least four fields in each image were analyzed. The fluorescence intensity was analyzed by ImageProPlus software 6.0 (National Institutes of Health, Bethesda, MD, USA).

**Reverse transcription-quantitative polymerase chain reaction (RT-qPCR).**
Total RNA was extracted from the adrenal tissues using a TRIzol kit (Thermo Fisher Scientific, Inc.). RNA concentrations were determined spectrophotometrically, and 1 μg total RNA was reverse transcribed into cDNA using an Avian Myeloblastosis Virus Reverse-Transcriptase kit (Promega Corporation, Madison, WI, USA). RT-qPCR was performed using the TB GreenTM Fast qPCR Mix (Takara Biotechnology Co., Ltd., Dalian, China). The following PCR primer sequences were used (5’–3’):

α7nAChR-F: ACAACATCTGCCAACACG,

α7nAChR-R: TTTCCCAACCTTTCTCCC;

β-actin-F: ATCGTCCACCGTAAATGC,

β-actin-R: TGAAGTGGTAGTCGGGTG.

The amplification reactions were performed with an Applied Biosystems 7500 Real-Time PCR System (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA), with initial denaturation at 95°C for 3 min, followed by 40 cycles of a two-step PCR at 95°C for 10 s, 53°C for 30 s, and 72°C for 30 s. The 2-ΔΔCt method was used to determine the amount of target, normalized to the endogenous reference, β-actin, as previously described (18).

**Western blotting.** Proteins were extracted from adrenal tissues as previously described (19) using a protein isolation kit (ReadyPrep; GE Healthcare Life Sciences). Protein concentration was determined using a bicinchoninic assay kit (Thermo Fisher Scientific, Inc.). A total of 20-μg protein was loaded into each lane and separated via SDS-PAGE on a 12% gel and transferred onto nitrocellulose membranes. Subsequently, membranes were blocked in 5% skim milk for 2 h in room temperature and incubated with the following primary antibodies overnight at 4°C: α7nAChR (1:1,000; Proentech, USA) and anti-β-actin (1:1,000; cat. no. 4970; Cell Signaling Technology, Inc., Danvers, USA). The nitrocellulose membranes were washed three times and incubated with HRP-labeled goat anti-rabbit IgG secondary antibody (1:10,000, cat. no. A16104SAMPLE; Thermo Fisher Scientific, Inc.) at 4°C for 2 h. Protein bands were visualized using an enhanced chemiluminescence kit (Thermo Fisher Scientific, Inc.), and the blots were scanned using a ChemiDoc XRS (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Protein expression was normalized to β-actin, and densitometric analysis was performed by ImageJ Software version 7.0 (National Institutes of Health, Bethesda, MD, USA).

**ELISA**

Serum adrenaline and norepinephrine (NE) were detected using ELISA, according to the manufacturer’s instructions (Adrenaline: abx514232, ABCAM; NE: KA3836, Abnova). The absorbance (OD value) of each well was measured at 450 nm. The measurements were carried out within 15 min.

**Statistical analysis**
Data were presented as the mean ± standard error of the mean and analyzed using SPSS version 17.0 (SPSS, Inc., Chicago, IL, USA). Significant differences were determined using one-way analysis of variance followed by the Bonferroni’s test. P<0.05 was considered to indicate a statistically significant difference.

Results

*Sh-a7nAChR lentivirus was established*

As shown in Figure 1, expression of α7nAChR was significantly down-regulated in 293T cells by all three interference sequences compared with the control group, and the effect of sh-α7nAChR1 was the most obvious. Therefore, sh-α7nAChR1 was packaged into lentiviruses. Briefly, 1-mL virus solution was diluted to a final titer of 1.0*10^8 TU/mL.

*a7nAChR expression was promoted in the adrenal tissues after RSV infection, but down-regulated by sh-a7nAChR lentivirus and/or DXMS*

Initially, HE staining was only carried out in normal control and model groups to ensure the success of the modeling. In the later experiments, we did not do the experiment in other groups. Compared with the control group, rat adrenal tissues with RSV infection showed obvious infiltration (Fig. 2A). As shown in Fig. 2B, the expression of α7nAChR at the mRNA level in the model group was higher than in the control group (P<0.05). The expression of α7nAChR in the sh-α7nAChR lentivirus, NGF antibody, DXMS, sh-α7nAChR lentivirus+NGF antibody, and sh-α7nAChR lentivirus+DXMS groups was significantly lower than that in the model group (P<0.05). Consistent with the mRNA expression, the protein expression of α7nAChR was also reduced in the sh-α7nAChR lentivirus, NGF antibody, DXMS, sh-α7nAChR lentivirus+NGF antibody, and sh-α7nAChR lentivirus+DXMS groups compared with the model group (P<0.05) (Fig. 2C). Importantly, α7nAChR expression at both mRNA and protein levels were significantly reduced in the sh-α7nAChR lentivirus+NGF antibody and sh-α7nAChR lentivirus+DXMS groups compared with the sh-α7nAChR lentivirus group.

*Silencing of α7nAChR reduced chromaffin granules and synaptic vesicles*

In the model group, cell membranes were shrunken and showed clubbed and villous processes; chromaffin granules were uniformly distributed; the contents of granules were fewer, and more vesicles were observed (Figure 3). By contrast, the cell membranes were also shrunken in the sh-α7nAChR group, while chromaffin granules were obviously reduced. The number of chromaffin granules and synaptic vesicles decreased in the anti-NGF treatment group. The distribution of chromaffin granules was slightly sparse in the DXMS treatment group. In the sh-α7nAChR+DXMS group, the nuclear membrane was shrunken. In addition, the mitochondria shrank slightly; the number of chromaffin granules and synaptic vesicles obviously decreased.

*Silencing of α7nAChR reduced the expression of synaptophysin and NF-κB*
As shown in Figs. 4 and 5, the expression of synaptophysin and NF-κB in the model group was significantly higher than that in the control group (P<0.05), while the expression of synaptophysin and NF-κB was significantly lower in the sh-α7nAChR lentivirus, NGF antibody, DXMS, sh-α7nAChR lentivirus+NGF antibody, and sh-α7nAChR lentivirus+DXMS groups than that in the model group (P<0.05). Importantly, the expression of synaptophysin and NF-κB was significantly reduced in the sh-α7nAChR lentivirus+NGF antibody and sh-α7nAChR lentivirus+DXMS groups compared with the sh-α7nAChR lentivirus group.

**Silencing of α7nAChR reduced serum adrenaline levels**

Compared with the control group, the model group showed decreased levels of serum adrenaline (P<0.05). Compared with the model group, the sh-α7nAChR lentivirus, sh-α7nAChR lentivirus+NGF antibody, and sh-α7nAChR lentivirus+DXMS groups showed significantly decreased serum adrenaline level (P<0.05). By contrast, single treatment with anti-NGF or DEX did not alter the adrenaline levels (vs. model, P>0.05, Fig. 6A). As shown in Fig. 6B, serum norepinephrine levels were not significantly altered in any group (P>0.05).

**Discussion**

In this study, we demonstrated that α7nAChR expression was elevated in asthmatic rats. The expression of α7nAChR was reduced by sh-α7nAChR virus, NGF antibody, and DXMS. Moreover, the chromaffin granules and synaptic vesicles were accordingly reduced by α7nAChR silencing. Moreover, the effects of α7nAChR silencing were promoted by NGF antibody or DXMS.

nAChR subunits, primarily the α7nAChR has been reported to redistribute in asthmatic mice (14, 15). Especially, α7nAChR was a potential therapeutic target for the treatment of ILC2s-dependent asthma (16). Nevertheless, our present study would further support the function of α-7nAChR in asthma using a different model. Especially, both pharmacological and genetic methods have been utilized in this study. First, the rat asthmatic model was established upon RSV infection in our study. The expression of α7nAChR increased in asthmatic rats and decreased after treatment with sh-α7nAChR viruses, anti-NGF antibody, and DXMS. These results further supported that NGF antibody suppressed the expression of α7nAChR in chromaffin cells caused by RSV infection (20). Second, the results of transmission electron microscopy further revealed that the numbers of chromaffin cells and synaptic vesicles increased after repeated RSV infection, while these numbers decreased after silencing of α7nAChR and anti-NGF antibody and DXMS treatment. The present study also implicated that occurrence of asthma was related to the increase of chromaffin cells and synaptic vesicles.

Inflammation or stimulation may elicit nerve terminals in the airway to release tachykinin, causing neurogenic inflammation and increased susceptibility to afferent nerves, respiratory changes, and cough (21). Our data also showed that the expression of synaptophysin after RSV infection increased significantly. However, silencing of α7nAChR and treatment with anti-NGF and DXMS decreased the
expression. As synaptophysin is an important structural protein of sensory neurons (22), the increase of
synaptophysin indicates changes to plasticity in nerve terminals.

The release of adrenaline from chromaffin cells depends on the activation of calcium channels to
produce sufficient concentration of \([\text{Ca}^{2+}]\), whereas \([\text{Ca}^{2+}]\) mainly depends on the regulation of \(\text{Ca}^{2+}\)
channels by subtypes of nAChR on chromaffin cell membranes (15). Previous studies have confirmed
that the \(\text{Ca}^{2+}\) permeability of different subtypes of nAChR is different (23-25). The nAChR subtype
composed of alpha 7–9 subunits has a higher \(\text{Ca}^{2+}\) permeability, while the \(\alpha7\text{nAChR}\) subtype has the
highest \(\text{Ca}^{2+}\) permeability (26). The \(\alpha7\text{nAChR}\) subtypes of chromaffin cells are regulated by NGF and
glucocorticoids (27). To maintain the endocrine phenotype of chromaffin cells, appropriate concentration
of glucocorticoids in the physiological state can inhibit or reduce the unnecessary release of adrenaline
by inhibiting the activation of \(\text{Ca}^{2+}\) channels (28).

Acetylcholine is released when sympathetic nerves projecting into the adrenal medulla are stimulated
(29). Thereafter, acetylcholine binds to the receptor and activates nAChR in chromaffin cells (30).
Activated calcium channels promote calcium influx, resulting in a significant increase of intracellular
calcium concentration, which triggers the release of catecholamines such as adrenaline, dopamine, and
norepinephrine (31). In this study, we also detected serum levels of adrenaline and norepinephrine.
Adrenaline, but not norepinephrine, level was reduced after RSV infection. Compensatory increase of
adrenaline release is required in the body. Over time, the compensatory release of adrenaline by
chromaffin cells gradually weakens, resulting in decreased adrenaline release; on the other hand, reduced
adrenaline levels after silencing of \(\alpha7\text{nAChR}\) is due to disorder of the hypothalamus pituitary adrenal
(HPA) axis in asthma (32).

During the course of asthma after RSV infection, the synthesis of adrenaline and the release process of
chromaffin cells were abnormal (33). After repeated infection with RSV, increase of NGF concentration
and decrease of glucocorticoid concentration induced the transformation of chromaffin cells from
endocrine phenotype to neuronal phenotype, resulting in the decrease of adrenaline synthesis (34). It may
be through a negative feedback mechanism that NGF induced the increase of \(\alpha7\text{nAChR}\) expression in
chromaffin cells. The decrease of cell threshold and abnormal release of adrenaline result in insufficient
secretion of adrenaline, provoking asthma.

Our study has some limitations. First, although 293 cells were an approved cell tool to evaluate the
efficiency of gene silencing, the more specific respiratory cell lines would strengthen our conclusion.
Moreover, the mechanisms could also be deeply investigated using the cultured respiratory cell lines.
Second, pathological changes of adrenal tissues (shown by HE staining) were only confirmed in the
model group. Whether \(\alpha7\text{nAChR}\) is involved in the pathological changes remains an important question.
In our future study, we will detect the pathological changes of all groups using HE staining. Third, it might
be also interesting to verify whether sh-\(\alpha7\text{nAChR}\) could repair the airway resistance. Fourth,
synaptophysin and NF-\(\kappa\text{B}\) expression will be further checked using western blotting. Moreover, its clinical
application also warrants more pharmacological and toxicological studies.
Conclusion

α7nAChR was elevated in rats after asthmatic modeling with RSV infection. The expression of α7nAChR was eliminated by sh-α7nAChR lentivirus, NGF antibody, and DXMS. Importantly, the silencing of α7nAChR reduces the number of chromaffin granules and synaptic vesicles and adrenaline release.

Declarations

Acknowledgements

Not applicable.

Funding

This study was supported by the National Natural Science Foundation of China (No:81460004 and 31960143); the JiangxiProvincial Cultivation Program for Academic and Technical Leaders of Major Subjects(No:20172BCB22025);Health and Family Planning Commission Traditional Chinese Medicine Foundation of Jiangxi Province(No:20165001).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors’ contributions

JW, ZFW and XZ performed the experiments and analyzed the data. JW and LXD designed the study and wrote the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Experimental protocols were approved and supervised by the Animal Care and Use Committee of People's Hospital of Nanchang University (No. 2019093).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests

Authors’ information (optional)

Not applicable.
References

1. Shimoda T, Obase Y, Nagasaka Y, Nakano H, Kishikawa R, Iwanaga T: Lung Sound Analysis and Airway Inflammation in Bronchial Asthma. The journal of allergy and clinical immunology. In practice 4: 505–511, 2016.

2. Altman MC, Reeves SR, Parker AR, et al. Interferon response to respiratory syncytial virus by bronchial epithelium from children with asthma is inversely correlated with pulmonary function. J Allergy Clin Immunol. 2018;142:451–9.

3. Wu X, Zhou X, Hu Y, Liu C, Wang J. Neutralization of nerve growth factor (NGF) inhibits the Th2 response and protects against the respiratory syncytial virus (RSV) infection. Immunol Res. 2017;65:721–8.

4. van Buul AR, Taube C. Treatment of severe asthma: entering the era of targeted therapy. Expert Opin Biol Ther. 2015;15:1713–25.

5. Agac D, Gill MA, Farrar JD. Adrenergic Signaling at the Interface of Allergic Asthma and Viral Infections. Frontiers in immunology. 2018;9:736.

6. Abroug F, Dachraoui F, Ouanes-Besbes L. Our paper 20 years later: the unfulfilled promises of nebulised adrenaline in acute severe asthma. Intensive care medicine. 2016;42:429–31.

7. Roberts G. Peanut allergy, anaphylaxis, adrenaline and exacerbations of asthma. Clinical experimental allergy: journal of the British Society for Allergy Clinical Immunology. 2016;46:1504–5.

8. Moroi K, Sato T. Comparison between procaine and isocarboxazid metabolism in vitro by a liver microsomal amidase-esterase. Biochem Pharmacol. 1975;24:1517–21.

9. Triaca V, Sposato V, Bolasco G, et al. NGF controls APP cleavage by downregulating APP phosphorylation at Thr668: relevance for Alzheimer's disease. Aging cell. 2016;15:661–72.

10. Zhao YZ, Jiang X, Xiao J, et al. Using NGF heparin-poloxamer thermosensitive hydrogels to enhance the nerve regeneration for spinal cord injury. Acta biomaterialia. 2016;29:71–80.

11. Yin J, Wang Y, Hu H, et al. P2X7 receptor inhibition attenuated sympathetic nerve sprouting after myocardial infarction via the NLRP3/IL−1beta pathway. J Cell Mol Med. 2017;21:2695–710.

12. Li X, Shaqura M, Mohamed D, et al. Pro- versus Antinociceptive Nongenomic Effects of Neuronal Mineralocorticoid versus Glucocorticoid Receptors during Rat Hind Paw Inflammation. Anesthesiology. 2018;128:796–809.

13. Streian C, Atanasescu I, Gavrilescu M. [Post-traumatic hemorrhagic subacute constrictive pericarditis]. Revista de medicina interna, neurologie, psihiatrie, neurochirurgie, dermato-venerologie. Medicina interna. 1975;27:129–33.

14. Chen X, Feng J, Hu C, Qin Q, Li Y, Qin L. Redistribution of adrenomedullary nicotinic acetylcholine receptor subunits and the effect on circulating epinephrine levels in a murine model of acute asthma. Int J Mol Med. 2017;39:337–46.

15. Wang J, Wang Y, Guo F, Feng Z, Wang X, Lu C. Nicotinic modulation of Ca2 + oscillations in rat cortical neurons in vitro. American journal of physiology Cell physiology. 2016;310:C748–54.
16. Galle-Treger L, Suzuki Y, Patel N, et al. Nicotinic acetylcholine receptor agonist attenuates ILC2-dependent airway hyperreactivity. Nature communications. 2016;7:13202.

17. Han M, Rajput C, Ishikawa T, Jarman CR, Lee J, Hershenson MB. Small Animal Models of Respiratory Viral Infection Related to Asthma. Viruses 102018.

18. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2^{(-ΔΔC(T))} Method. Methods. 2001;25:402–8.

19. Li J, Yang S, Zhu G. Postnatal calpain inhibition elicits cerebellar cell death and motor dysfunction. Oncotarget. 2017;8:87997–8007.

20. Safieh-Garabedian B, Oz M, Bey RM, et al. Involvement of the alpha7-nicotinic acetylcholine receptors in the anti-inflammatory action of the thymulin-related peptide (PAT). Neuroscience. 2013;250:455–66.

21. Satheeshkumar PS, Mohan MP. Tachykinin peptide, substance P, and its receptor NK–1R play an important role in alimentary tract mucosal inflammation during cytotoxic therapy. Digestive diseases sciences. 2014;59:2864–73.

22. Sun J, Zhu G, Liu Y, et al. UBE3A Regulates Synaptic Plasticity and Learning and Memory by Controlling SK2 Channel Endocytosis. Cell reports. 2015;12:449–61.

23. Uteshev VV. Evaluation of Ca2 + permeability of nicotinic acetylcholine receptors in hypothalamic histaminergic neurons. Acta Biochim Biophys Sin. 2010;42:8–20.

24. Adams DJ, Nutter TJ. Calcium permeability and modulation of nicotinic acetylcholine receptor-channels in rat parasympathetic neurons. J Physiol. 1992;86:67–76.

25. Zhou Z, Neher E. Calcium permeability of nicotinic acetylcholine receptor channels in bovine adrenal chromaffin cells. Pflug Arch: Eur J Physiol. 1993;425:511–7.

26. Safronova VG, Vulfius CA, Shelukhina IV, et al. Nicotinic receptor involvement in regulation of functions of mouse neutrophils from inflammatory site. Immunobiology. 2016;221:761–72.

27. Carrasco-Serrano C, Criado M. Glucocorticoid activation of the neuronal nicotinic acetylcholine receptor alpha7 subunit gene: involvement of transcription factor Egr–1. FEBS Lett. 2004;566:247–50.

28. Harada K, Matsuoka H, Fujihara H, Ueta Y, Yanagawa Y, Inoue M. GABA Signaling and Neuroactive Steroids in Adrenal Medullary Chromaffin Cells. Frontiers in cellular neuroscience. 2016;10:100.

29. Hsieh JT, Kuo YC, Chang HC, Liu SP, Chen JH, Tsai VF. The role of sympathetic and parasympathetic nerve systems on the smooth muscle of rat seminal vesicles - experimental results and speculation for physiological implication on ejaculation. Andrology. 2014;2:59–64.

30. Heinemann S, Bevan S, Kullberg R, Lindstrom J, Rice J. Modulation of acetylcholine receptor by antibody against the receptor. Proceedings of the National Academy of Sciences of the United States of America 74: 3090–3094, 1977.

31. Felix-Martinez GJ, Gil A, Segura J, Villanueva J, Gutierrez LM. Modeling the influence of co-localized intracellular calcium stores on the secretory response of bovine chromaffin cells. Comput Biol Med.
32. Hotta H, Watanabe N, Piche M, Hara S, Yokawa T, Uchida S. Non-noxious skin stimulation activates the nucleus basalis of Meynert and promotes NGF secretion in the parietal cortex via nicotinic ACh receptors. J Physiol Sci. 2014;64:253–60.

33. Piedimonte G, Perez MK. Respiratory syncytial virus infection and bronchiolitis. Pediatrics in review. 2014;35:519–30.

34. Feng JT, Wu XM, Li XZ, Zou YQ, Qin L, Hu CP. Transformation of adrenal medullary chromaffin cells increases asthmatic susceptibility in pups from allergen-sensitized rats. Respiratory research. 2012;13:99.

### Tables

#### Table 1

Sequences of sh-α7nAChR.

| Genes     | Sequences (5′–3′)                           |
|-----------|--------------------------------------------|
| α7nAChR−1-F | CACCGGTGAATGCATCTGGGCATTGCGAACAATGCCCCAGATGCATTCCACC |
| α7nAChR−1-R | AAAAGGTGAATGCATCTGGGCATTGTTCGCAATGCCCCAGATGCATTCCACC |
| α7nAChR−2-F | CACCGCAGTGCAAACTGAAGTTTGGCGAACCAAACTTCAGTTTGCACTGC |
| α7nAChR−2-R | AAAAGCAGTGCAAACTGAAGTTTGGCGAACCAAACTTCAGTTTGCACTGC |
| α7nAChR−3-F | CACCGCAGATATCAGCAGCTATATCCGAAGATATAGCTGCTGATATCTGC |
| α7nAChR−3-R | AAAAGCAGATATCAGCAGCTATATCCGAAGATATAGCTGCTGATATCTGC |

### Figures
sh-α7nAChR down-regulates α7nAChR expression in 293T cells. Three sh-α7nAChR sequences were established and sh-α7nAChR-1 showed the best silencing effect. *P<0.05 compared with control (Bonferonni’s test).

Figure 1
α7nAChR expression is promoted in an RSV infection-induced asthmatic rat model, while it is down-regulated by sh-α7nAChR lentivirus. A) The asthmatic rat model was confirmed by HE staining (x200). Obvious inflammation was displayed in the model group. B) mRNA expression of α7nAChR in each group. C) Protein expression of α7nAChR in each group. Upper panel: representative blots; Lower panel:
quantification data. *P<0.05 compared with control; #P<0.05 compared with the model group (Bonferonni’s test).

Figure 3

α7nAChR expression is promoted in an RSV infection-induced asthmatic rat model, while it is down-regulated by sh-α7nAChR lentivirus. A) The asthmatic rat model was confirmed by HE staining (x200). Obvious inflammation was displayed in the model group. B) mRNA expression of α7nAChR in each group. C) Protein expression of α7nAChR in each group. Upper panel: representative blots; Lower panel: quantification data. *P<0.05 compared with control; #P<0.05 compared with the model group (Bonferonni’s test).
Figure 4

The silencing of α7nAChR reduced expression of synaptophysin. The expression of synaptophysin in the model group was significantly higher than that in the control group, and the expression of synaptophysin and NF-κB was significantly lower in the sh-α7nAChR lenti-virus, NGF antibody, DXMS, sh-α7nAChR lenti-virus+NGF antibody, and sh-α7nAChR lenti-virus+DXMS groups. *P<0.05 compared with control; #P<0.05 compared with model group (Bonferonni’s test). Scale bar: 100 μm.
The silencing of α7nAChR reduced expression of synaptophysin. The expression of synaptophysin in the model group was significantly higher than that in the control group, and the expression of synaptophysin and NF-κB was significantly lower in the sh-α7nAChR lenti-virus, NGF antibody, DXMS, sh-α7nAChR lenti-virus+NGF antibody, and sh-α7nAChR lenti-virus+DXMS groups. *P<0.05 compared with control; #P<0.05 compared with model group (Bonferonni’s test). Scale bar: 100 μm.
Figure 6

The silencing of α7nAChR reduced serum adrenaline. A) Adrenaline level; B) Norepinephrine level. *P<0.05 compared with control; #P<0.05 compared with model group; +P<0.05 compared with anti-NGF group; -P<0.05 compared with DEX group (Bonferonni’s test).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Figure.pptx