Role of 5-HT_{2A} Receptors in the Depression of Respiratory Rhythmic Discharge Activity of Medullary Slice of Neonatal Rats with Prenatal Cigarette Smoke Exposure

M Ji, Y Wang, Z Qian

Affiliation:
College of Basic Medical Science, Xinxiang Medical University, Xinxiang 453003, China

Correspondence:
Professor Z Qian
College of Basic Medical Science
Xingxiang Medical University
Xinxiang 453003
Henan Province
China
E-mail: zhibinqiancn@163.com

Short title: 5-HT_{2A} Receptors Decline with Cigarette Smoke Exposure

Synopsis: This study investigated the role of 5-HT_{2A} receptors in the depression of respiratory rhythmic discharging activity of medullary slice of neonatal rats with prenatal cigarette smoke exposure (PCSE), the agonists and antagonists of 5-HT_{2A} receptors were applied to isolated medullary slices of neonatal rats.

ABSTRACT

Objective: Prenatal cigarette smoke exposure (PCSE) is associated with numerous neurodevelopmental abnormalities such as respiratory depression. Although it is clear that 5-HT_{2A} receptors play an important role in the generation and regulation of respiratory rhythm, but the role of 5-HT_{2A} receptors in the depression of respiratory rhythmic discharge activity (RRDA) of medullary slice of neonatal rats with prenatal cigarette smoke exposure is not well understood.

Methods: 1-(2, 5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), a 5-HT_{2A} receptor agonist, could excite RRDA of control group and experimental group, and the effects of DOI on control group was stronger than those on experimental group; ketanserine, a 5-HT_{2A} receptor antagonist, had inhibitory effects on RRDA of the two groups, and the inhibitory effects on control group was stronger than those on experimental group. The western blotting and RT-qPCR technology were used to detect whether 5-HT_{2A} receptors protein and 5-HT_{2A} receptors mRNA would be changed or not.

Results: We found that the expression of 5-HT_{2A} receptors protein were down-regulation, and the expression of 5-HT_{2A} receptors mRNA had not changed. **Conclusion:** The possible mechanism of PCSE inhibition respiratory rhythmic discharge activity in the medullary slice of neonatal rats is the decrease of the function of 5-HT_{2A} receptors and down-regulation of protein expression.

Keywords: prenatal cigarette smoke exposure (PCSE), medullary slice, 5-HT_{2A} receptors, respiratory rhythmic discharge activity (RRDA)

INTRODUCTION

In modern society, cigarette smoking has become a problem that can not be ignored. Tobacco smoke contains various bioactive compounds which can produce adverse effects on smokers as well as on surrounding crowds, especially on pregnant women. Prenatal cigarette smoke exposure (PCSE) is associated with an increase in infant morbidity and mortality (1). The blood concentrations of nicotine and nicotine metabolites induced by PCSE in the fetus and infant are equal or even higher than those of the smoking mother (2, 3). The long-term adverse effects of PCSE could carry a lot of serious complications such as preterm delivery, intrauterine growth retardation, stillbirth, congenital malformations, low birth weight, respiratory diseases, and increased risk of sudden infant death syndrome (SIDS). In the fetal development period, smoking during pregnancy can make fetal vasoconstriction and hypoxia (4). Carbon monoxide in the smoke can enter into the blood through the placental barrier, which leads to the increased carboxyhemoglobin level of umbilical cord blood, which in turn inhibits the release of oxygen into the fetal tissues (5). This chronic hypoxia may change the physiological development of tissues and organs, especially those most susceptible areas to hypoxic damage, such as the central nervous system (6). Acute nicotine exposure impaired central CO_2 response, and attenuated respiratory drive (7). Chronic prenatal nicotine exposure reduced frequency of the respiratory discharge of slices and the ability of the slice to maintain a respiratory rhythm during expose to severe hypoxia (8).

5-HT is an important neurotransmitter in the central nervous system, which promotes brain development (9), and participates in the modulation of central respiratory activity (10, 11). The activation of 5-HT_{2A} receptors in mammals is associated with the generation and

regulation of respiratory rhythm (12), and gasping activity is dependent on 5-HT_{2A} receptors during hypoxia (13). Moreover, it has been proved that the abnormal distribution of 5-HT_{2A} receptors could lead to SIDS and congenital central hypoventilation syndrome (CCHS) (14).

To investigate the role of 5-HT_{2A} receptors in the depression of respiratory rhythmic discharging activity of medullary slice of neonatal rats with PCSE, the agonists and antagonists of 5-HT_{2A} receptors were applied to isolated medullary slices of neonatal rats. In addition, western blotting and RT-qPCR technology were also used to observe whether 5-HT_{2A} receptors protein and mRNA expression would be changed or not.

MATERIALS AND METHODS

Animals

Sprague-Dawley adult rats were obtained from the Laboratory Animal Center of Zhengzhou University. All experimental protocols were approved by the Institutional Animal Care and Use Committee at the Xinxiang Medical University. Neonatal rats (1~3 days old), both sexes, were used for the present study. The neonatal rats of control group were delivered by adult rats which were fed routinely; the neonatal rats of PCSE group were delivered by adult rats which were fed routinely and exposed to smoke from mating. The adult rats were put into smoke box (80 cm * 60 cm * 100 cm) to smoke 30min by 8 cigarettes twice daily (8 AM and 8 PM) from mating to delivery. All animals were maintained at room temperature 27-28℃ under a 12 h light/12 h dark cycle.

Electrophysiological studies

The brainstem spinal cord was obtained as follow. Rats were deeply anesthetized with ether

until the nociceptive reflexes were abolished and quickly decapitated at the C3–C4 spinal level. The brainstem was isolated in ice - cold artificial cerebrospinal fluid (ACSF) containing (in mM): 124 NaCl, 5 KCl, 1.2KH_2PO, 2.4 $CaCl_2$, 1.3 $MgSO_4$, 26 $NaHCO_3$ and 30 glucose equilibrated with carbogen (95% O_2 and 5% CO_2) at PH 7.4 and the process was ended in 3 min. After dorsal side was glued on an agar block, the brainstem was sectioned serially in the transverse plane until inferior olive appeared which indicated that the rostral boundary of the medial area of nucleus retrofacialis (mNRF) would appear, then a single transverse slice containing mNRF was cut (an 850 μm thick). The brainstem slice was quickly transferred to a recording chamber and continuously perfused with oxygen-saturated ACSF at a rate of 4–6 ml/min, and temperature was maintained at 27–29℃. The discharge of hypoglossal nerve rootlets was recorded by suction electrodes. Signals were amplified and band-pass filtered (100 Hz–3.3 kHz), and data were sampled (5 kHz) and stored in the computer via BL-420 biological signal processing system. In electrophysiological studies, 1-(2, 5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), a 5-HT_{2A} receptor agonist, and Ketanserine, a 5-HT_{2A} receptor antagonist were applied, and those slices in control group were randomly divided into 3 groups (n=6 for each): control blank group, control DOI group, control Ketanserine group; and the PCSE group were also randomly divided into 3 groups (n=6 for each): PCSE blank group, PCSE DOI group, PCSE Ketanserine group. DOI and Ketanserine were purchased from Sigma (Sigma Co., St. Louis, MO, USA). Other chemicals were of analytical grade. In control blank group and PCSE blank group, slices were perfused with ACSF. In control DOI group and PCSE DOI group, slices were perfused with ACSF which contained 40 μmol/L DOI after perfusion with ACSF for 10 min. In control

Ketanserine group and PCSE Ketanserine group, slices were perfused with ACSF which contained 40 μmol/L Ketanserine after perfusion with ACSF for 10 min.

Western blotting

Protein was extracted from the medulla oblongata segments (at the level of mNRF). Concentration of proteins in the supernatant was determined with a bicinchoninic acid (BCA) protein quantification kit according to the instructions. Briefly, these equal amounts of samples (50 μg of protein per lane) were loaded on 10% SDS-polyacrylamide gels for electrophoresis and then electrotransferred onto polyvinylidene difluoride (PDVF) membranes (Millipore, Billerica, MA, USA). The membranes were blocked with 5% bovine serum albumin diluted in Tris-Buffered Saline Tween-20 for 1h, followed by incubation with primary antibodies (goat polyclonal antibody against 5-HT2A receptor, 1:100, Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 4 °C overnight. After washing, the membranes were incubated with secondary antibodies (HRP AffiniPure Rabbit Anti-Goat IgG, 1:2000, EarthOx, USA) for 1 h at room temperature. GAPDH (the primary antibody from Cell Signaling Technology, 2118L, Beverly, MA, USA, working concentration : 1:1000) was blotted in the same membrane as an internal control for normalizing the relative density. Immunoreactive bands were visualized by enhanced chemiluminescence (ECL, Amersham; Piscataway, NJ), and then quantified by imageJ software (Molecular Dynamics IQ solutions, Molecular Dynamics, Inc., Sunnyvale, CA).

RT-qPCR

Total RNA was extracted from mNRF area using the RNAiso Plus (TaKaRa, Dalian, China) following the manufacturer recommended protocol. The primer sequences for rat 5-HT_{2A} receptor are: forward 5'-AAGCTGCAGAATGCCACCAAC-3', and reverse 5'-CCAGGTAAATCCAGACGGCACA-3'; and for rat GAPDH are: forward 5'-GGCACAGTCAAGGCCGAGAATG-3', and reverse 5'-ATGGTGGTGAAGACACCAGTA-3'. Real-time RT-qPCR was performed in a final volume of 50 µl. 1 µl of sample RNA, 25 µl Premix Taq (contains: TaKaRa Taq 1.25 U/25 µl, dNTP Mixture 2*conc.; each 0.4 mM, PCR Buffer 2*conc.; 3mM Mg2+) (TaKaRa, Dalian, China), 1 µl forward primer, 1 µl reverse primer, and RNase free sterile water added to 50µl were amplified DNA. Cycling parameters were as follows:1 cycle at 95℃ for 30 second,1 cycle at 95℃ for 15s,1 cycle at 60℃ for 35s, followed by 40 cycles of denaturing and annealing/elongation. The transcript of a housekeeping gene, GAPDH, served as internal control and serial dilutions of the positive control were performed on each plate to create a standard curve. The amount of target gene was normalized to the reference GAPDH to obtain the relative threshold cycle.

Statistical analyses

SPSS 13.0 software (SPSSInc, Chicago, USA) was used to analyze data. All data were represented as mean ± S.E.M, and repeated measures of ANOVA and post-hoc test were used to compare the values obtained before and after drug application, and multiple comparison

between the groups was performed using S-N-K method. P<0.05 indicated that the difference was statistically significant.

RESULTS

RRDA of the control group compared with the PCSE group

Respiratory rhythmic discharge activity (RRDA) of the control group and PCSE group was stability without attenuation in 60 min, which illustrates that the experimental model is stable and reliable. In smoked group, compared with control group, PCSE could lead to the reduced inspiratory time (TI) (t=15.011, P= 0.004), the extend respiratory cycle (RC) (t=12.489, P=0.006) and the decreased integral amplitude (IA) (t=22.821, P=0.002) (Table 1; Figures 1, 2).

Effect of DOI on hypoglossal nerve rootlets RRDA of control group and PCSE group

One to two minutes after the application of DOI (40 μmol/L) to the bath both the frequency and integral amplitude of RRDA were increased; TI were extended (control group, t= 11.794, P=0.006; PCSE group, t=3.46, P=0.074); RC were shortened (control group, t=13.510, P=0.005; PCSE group, t=7.264, P=0.015); IA were increased (control group, t=39.925, P=0.001; PCSE group, t=35.674, P=0.000). The degree of RRDA changes in control group was higher than that in PCSE group (Table 2; Figure 3).

Effect of Ketanserine on hypoglossal nerve rootlets RRDA of control group and PCSE group

Perfused with ACSF which contained 40μmol/L Ketanserine, we found that TI (control group, t=21.651, P=0.002; PCSE group, t=12.990, P=0.006) and IA (control group, t=25.154, P=0.001; PCSE group, t=47.979, P=0.000) of RRDA in control group and PCSE group were decreased, and RC (control group, t=10.492, P=0.009; PCSE group, t=5.595, P=0.030) were extended. The degree of RRDA changes in control group was higher than that in PCSE group (Table 3; Figure 4).

Western Blotting

The expressions of 5-HT_{2A} receptors protein of the PCSE group were weaker than the control group (Figure 5).

RT-qPCR

There was not significant increase in the levels of mRNA encoding 5-HT_{2A} receptors as measured by the RQ values in the medullary slice of neonatal rats of control group compared to that of PCSE group (Figure 6).

DISCUSSION

The aim of the study was to investigate the correlation between respiratory activity depression induced by prenatal cigarette smoke exposure and 5-HT_{2A} receptors pathway. The slice, which contained the medial area of nucleus retrofacialis (mNRF) (15) that produce respiratory rhythmic discharge activity, can be used to study the respiratory function. In this experiment, we used electrodes record RRDA to reflect respiratory function of medullary,

used western blotting to study expression level of 5-HT_{2A} receptors and used RT-qPCR to study mRNA of 5-HT_{2A} receptors.

Cigarette smog contains over 4000 chemical constituents, while carbon monoxide and nicotine exposure are of large concern. 5-HT system plays a major role in early CNS growth and development, including the development of the medullary respiratory rhythm. Acute nicotine administration increased 5-HT release in the cortex, hippocampus, striatum, hypothalamus, dorsal raphe nucleus (DRN) and spinal cord (16); long-term application reduced 5-HT concentration and synthesis (17). Nicotine exposure decreased 5-HT (2) receptor binding in brain 5-HT projections regions, and altered the concentrations and functions of postsynaptic 5-HT receptors in a manner commensurate with impaired 5-HT synaptic function (18). And nicotine could affect 5-hydroxytryptamine system of brain stem and reduced the immunoreactivity of 5-HT receptors (19). 5-HT_{2A} receptors played a permissive role in the sensitizing effects of nicotine. Stimulation of 5-HT_{2A} receptors enhanced the development of nicotine sensitization (20). Nicotine could regulate respiratory rhythm and pattern (21). Smoking during pregnancy can make fetal hypoxia, which modulated 5-HT receptor density and agonist affinity (22), and our data also showed that smoking attenuated the expression of 5-HT_{2A} receptors.

In our study, PCSE shorten TI, extend RC and decrease IA of RRDA（Table 1）. Activation of 5-HT_{2A} receptors with DOI increased TI, IA and shortened RC in control group; and increased TI, IA and shortened RC in PCSE group. This showed agonist of 5-HT_{2A} receptors for 5-HT_{2A} receptors in PCSE group had excitation effects, but the excitation effects were lower than control group (Table 2). This illustrated that the reactivity of 5-HT_{2A}

receptors to DOI was reduced in PCSE group. Inhibition of 5-HT_{2A} receptors with ketanserine shortened TI, IA and extended RC in control group; and shortened TI, IA and extended RC in PCSE group. These pointed out antagonist of 5-HT_{2A} receptors for 5-HT_{2A} receptors in PCSE group had inhibitory effects, but the inhibitory effects were lower than control group (Table 3). This illustrated that the reactivity of 5-HT_{2A} receptors to ketansreine was reduced in PCSE group. Above two cases showed that repeatedly given cigarette smog could reduce the function of 5-HT_{2A} receptors. While after repeated cigarette smog, application of western blotting techniques found that 5-HT_{2A} receptors expression in protein levels in medulla oblongata of newborn rat were down-regulation; application of RT-qPCR technology found that 5-HT_{2A} receptor expression in transcription and translation levels had not changed. These demonstrated that repeated cigarette smog reduced the function of 5-HT_{2A} receptors by changing the protein expression not gene expression.

Chronic nicotine exposure desensitized nicotinic receptors and affected important ion channels genes expression, such as sodium, potassium, and calcium channels. Cigarette smoke induced cell damage through the following path: it first induces an increase in $[Ca^{2+}]i$ by activation intracellular Ca^{2+}; the increase in Ca^{2+} activates PKCαand PKCεthrough activation of Ca^{2+}-dependent signal transduction molecules; the PKCs activate NADPH oxidase to generate ROS (23). So we inferred that this way is one of reasons to cause 5-HT_{2A} receptors damage.

PKC is a key link in the process of signaling pathways and regulates neurotransmitters release, receptor desensitization, ion channel characteristics, etc. 5-HT_{2A} receptors link to the Gq family of G-proteins, and coupled to phospholipase C, which causes increase of

intracellular Ca^{2+} concentration and the activation of PKC. PKC direct phosphorylation of Na^{+} channel, which make the Na^{+} channel open, reduce the action potential absolute value and extend opening time. Additionally, PKC can increase reactive oxygen species (ROS) generation that may oxidize Na^{+} channel and make its conformation changes to increase Na^{+} currents. 5 - HT_{2A} receptors affect generation and regulation mechanism of the basic rhythmic respiration through PKC and ROS affect sodium current in respiratory neurons.

There are some cross regions between 5-HT_{2A} receptors signal transduction pathways and nicotinic receptors (16). Nicotine repeated action on nicotine receptors lead to them desensitization or inactivation, and the update rate is reduced (24). And nicotinic receptor desensitization can inhibit the activity of PKC (25). The mechanism of 5 - HT_{2A} receptors effect the generation and modulation of respiratory rhythm may be by modulating sodium conductance via a PKC pathway. Inhibition PKC activity could reduce the rate of Na^{+} channels open, augment action potential absolute value and shorten the opening hours. This mechanism may be inhibit 5 - HT_{2A} receptor function and is one of the main ways to produce respiratory depression.

ACKNOWLEDGEMENTS

This work was supported by Department of Functional Laboratory.

AUTHORS' NOTE

Conceived and designed the experiments: QXL. Performed the experiments: JML, WYX, QXL. Analyzed the data: JML, WYX, QXL. Contributed reagents/materials/analysis tools:

JML, WYX, QXL. Wrote the paper: JML, QXL. The authors declare that they have no conflicts of interest.

REFERENCES

Boucher O, Jacobson JL, Burden MJ, Dewailly É, Jacobson SW, Muckle G. Prenatal tobacco exposure and response inhibition in school-aged children: an event-related potential study. Neurotoxicol Teratol 2014; **44:** 81-8.

Berlin I, Heilbronner C, Georgieu S, Meier C, Spreux-Varoquaux O. Newborns' cord blood plasma cotinine concentrations are similar to that of their delivering smoking mothers. Drug Alcohol Depend 2010; **107:** 250-2.

Blood-Siegfried J, Rende EK. The long-term effects of prenatal nicotine exposure on neurologic development. J Midwifery Womens Health 2010; **55:** 143-52.

Rogers J M. Tobacco and pregnancy. Reprod Toxicol 2009; **28:** 152-60.

Mattsson K, Källén K, Rignell-Hydbom A, Hansson SR, McElrath TF, Cantonwine DE et al. Maternal Smoking during Pregnancy and Daughters' Preeclampsia Risk. PLoS One 2015; 10: **1-13.**

Heikkinen AM, Meurman JH, Sorsa T. Tobacco, snuff and oral health. Duodecim 2015; 131: **1975-80.**

Brundage CM, Cartagena CM, Potter EA, Taylor BE. Nicotine elicits a developmentally dependent depression in bullfrog neuroventilatory response to CO(2). Respir Physiol Neurobiol 2010; **170:** 226-35.

Hu H, Brahmbhatt A, Upadhyaya R, Vega D, Hill AA. Prenatal nicotine exposure alters the response of the mouse in vitro respiratory rhythm to hypoxia. Respir Physiol Neurobiol 2012; **181:** 234-47.

Bonnin A, Levitt P. Fetal, maternal, and placental sources of serotonin and new implications for developmental programming of the brain. Neuroscience 2011; **197:** 1-7.

Zygmunt M, Chłoń-Rzepa G, Sapa J. The effect of serotonin 5-HT1A, 5-HT2 receptor ligands, ketoprofen and their combination in models of induced pain in mice. Acta Pol Pharm 2015; **72:** 579-85.

Hodges MR, Richerson GB. The role of medullary serotonin (5-HT) neurons in respiratory control: contributions to eupneic ventilation, CO_2 chemoreception, and thermoregulation. J Appl Physiol 2010; **108:** 1425-32.

Pena F, Ramirez JM. Endogenous activation of serotonin-2A receptors is required for respiratory rhythm generation in vitro. J Neurosci, 2002, **22:** 11055-64.

Tryba AK, Pena F, Ramirez JM. Gasping activity in vitro: a rhythm dependent on 5-HT2A receptors. J Neurosci 2006; **26:** 2623-34.

Ozawa Y, Okado N. Alteration of serotonergic receptors in the brain stems of human patients with respiratory disorders. Neuropediatrics 2002; **33:** 142-9.

Wu ZH, Zhang FT, Li YR. Role of the medial region of nucleus retrofacialis of the rabbit in the generation of respiratory rhythm. Sheng Li Xue Bao 1990; **42:** 68-75.

Seth P, Cheeta S, Tucci S, File SE. Nicotinic--serotonergic interactions in brain and behaviour. Pharmacol Biochem Behav 2002; **71:** 795-805.

Benwell M E, Balfour D J. Effects of nicotine administration and its withdrawal on plasma corticosterone and brain 5-hydroxyindoles. Psychopharmacology (Berl) 1979; **63:** 7-11.

Xu Z, Seidler FJ, Cousins MM, Slikker W Jr, Slotkin TA. Adolescent nicotine administration alters serotonin receptors and cell signaling mediated through adenylyl cyclase. Brain Res 2002; **951:** 280-92.

Say M, Machaalani R, Waters KA. Changes in serotoninergic receptors 1A and 2A in the piglet brainstem after intermittent hypercapnic hypoxia (IHH) and nicotine. Brain Res 2007; **1152:** 17-26.

Zaniewska M, McCreary AC, Wydra K, Filip M. Differential effects of serotonin (5-HT)2 receptor-targeting ligands on locomotor responses to nicotine-repeated treatment. Synapse 2010; **64:** 511-9.

Shao XM, Feldman JL. Mechanisms underlying regulation of respiratory pattern by nicotine in preBotzinger complex. J Neurophysiol 2001; **85:** 2461-7.
Angeles DM, Williams J, Zhang L, Pearce WJ. Acute hypoxia modulates 5-HT receptor density and agonist affinity in fetal and adult ovine carotid arteries. Am J Physiol Heart Circ Physiol 2000; **279:** H502-H10.
Mai Y, Higashi T, Terada K, Hatate C, Nepal P, Horiguchi M et al. Nicotine- and tar-free cigarette smoke extract induces cell injury via intracellular Ca2+-dependent subtype-specific protein kinase C activation. J Pharmacol Sci 2012; **120:** 310-4.
Mc Donough M. Update on medicines for smoking cessation. Aust Prescr 2015; **38:** 106-11.
Sun X, Liu Y, Hu G, Wang H. Activities of cAMP-dependent protein kinase and protein kinase C are modulated by desensitized nicotinic receptors in the rat brain. Neurosci Lett 2004; **367:** 19-22.

Table 1: The effects of smoking on hypoglossal nerve rootlets RRDA ($\bar{x}$ ±s, n=6)

Group	**TI (s)**	**RC (s)**	**IA (μV·s)**
control group	1.03 ± 0.11	14.26 ±1.65	338.86 ± 15.63
PCSE group	0.77 ± 0.08**	18.37 ±2.12**	302.10 ±18.42**
control+DOI	1.21 ± 0.07**	11.33 ± 1.45**	397.28 ± 17.21**
PCSE+DOI	0.87 ± 0.05$^{\#}$	15.57 ± 1.78$^{\#}$	335.93 ± 11.13$^{\#\#}$
control+ Ketanserine	0.78 ± 0.09**	17.81 ± 2.07**	270.12 ± 22.73**
PCSE+Ketanserine	0.62 ± 0.06$^{\#\#}$	22.60 ± 2.12$^{\#}$	269.69 ± 17.25$^{\#\#}$

TI: Inspiratory time; RC: Respiratory cycle; IA: integral amplitude.

*P<0.05,**P<0.01 compared with control group, $^{\#}P$<0.05, $^{\#\#}P$<0.01 compared with PCSE group.

Table 2: Change rate of the effect of DOI on RRDA of control group and PCSE group ($\bar{x}$ ±s, n=6)

Group	**TI (%)**	**RC (%)**	**IA (%)**
(Control+DOI)/ Control	17.47±1.98	20.55±1.52	17.24±2.74
(PCSE+DOI)/ PCSE	12.99±1.14**	14.24±2.62*	11.19 ±1.06**
t	9.121	4.044	5.800
P	0.003	0.027	0.010

TI: Inspiratory time; RC: Respiratory cycle; IA: integral amplitude.

*P<0.05, **P<0.01 compared with control group + DOI.

Table 3: Change rate of the effect of Ketanserine on RRDA of control group and PCSE group ($\bar{x}$ ±s, n=6)

Group	**TI(%)**	**RC(%)**	**IA(%)**
(Control+Ketanserine)/ Control	24.27±3.49	24.89±3.33	20.29±2.95
(PCSE+Ketanserine)/ PCSE	19.48±2.66*	16.68±1.58*	10.73±1.59**
t	4.236	3.762	13.080
P	0.024	0.033	0.001

TI: Inspiratory time; RC: Respiratory cycle; IA: integral amplitude.

*P<0.05, **P<0.01 compared with control group + Ketanserine.

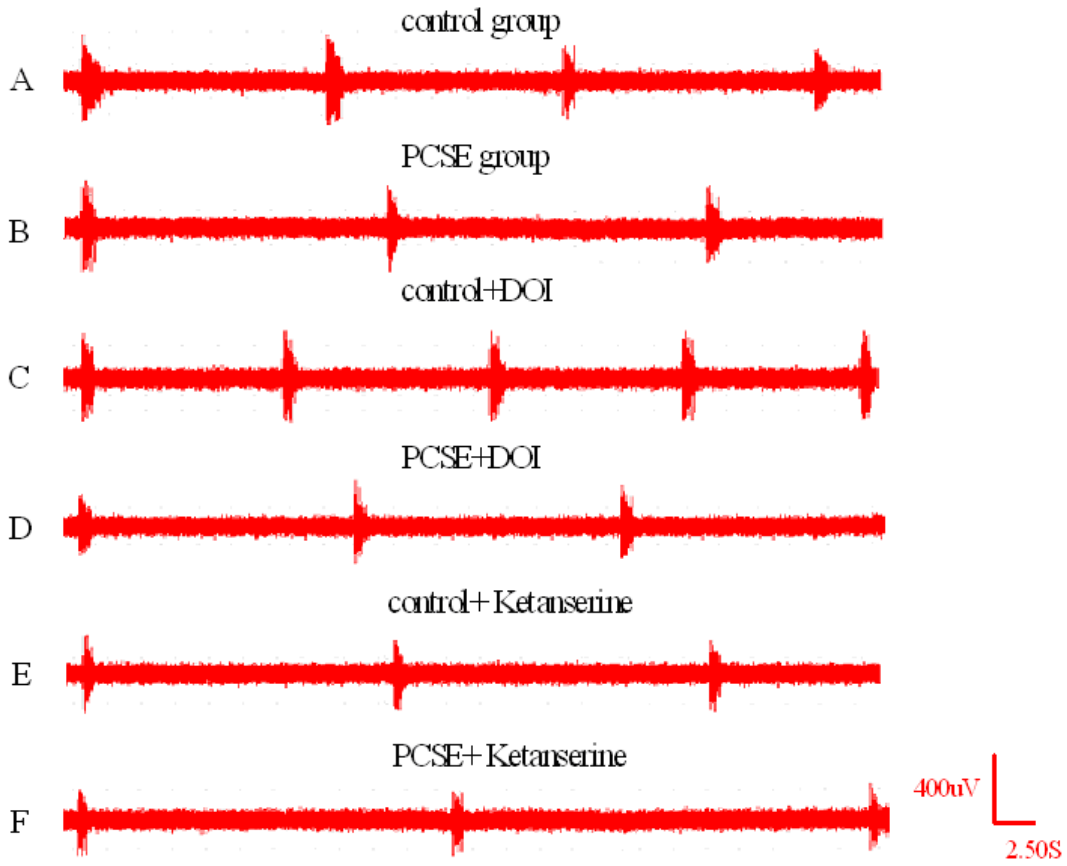


Fig. 1: The effects of DOI and Ketanserine on RRDA of control group and PCSE group. The upper six groups show the raw trace of hypoglossal nerve (Ⅻ) activity. Their discharge activities were recorded during perfusion with ACSF (A, B), after application of 40μmol/L DOI (C, D), after application of 40μmol/L Ketanserine (E, F).

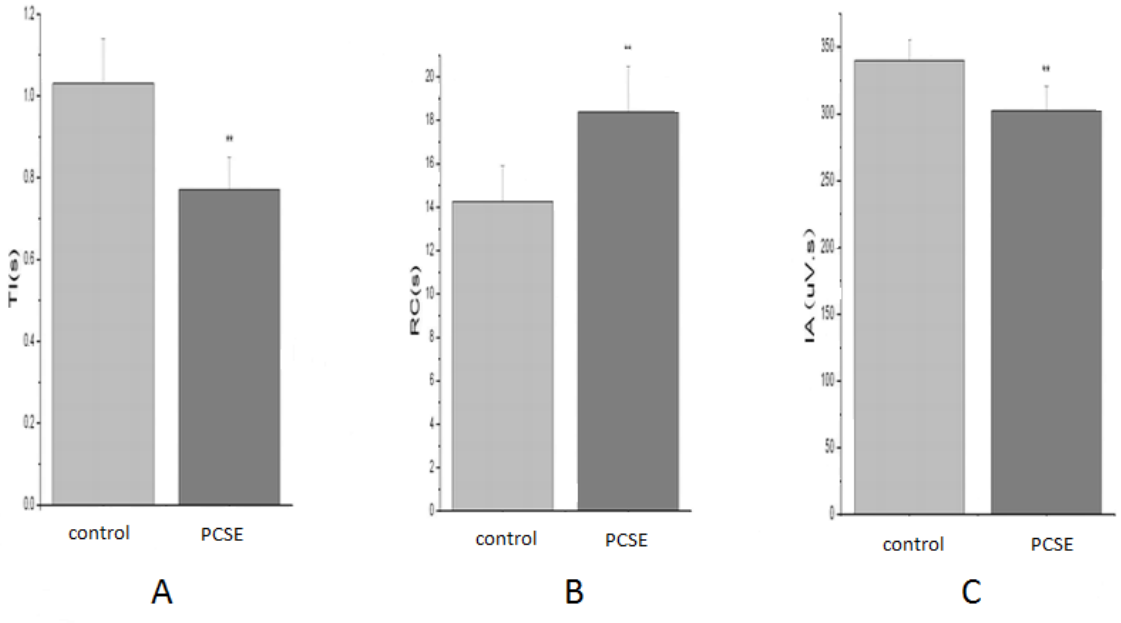


Fig. 2: RRDA of control group compared with that of PCSE group. (A) Inspiratory time of RRDA in slices from control group and PCSE group. (B) Respiratory cycle of RRDA in two groups. (C) Integral amplitude of RRDA in two groups. All values are mean ± S.E.M. * *P*<0.05 compared to control group, ***P*<0.01 compared to control group.

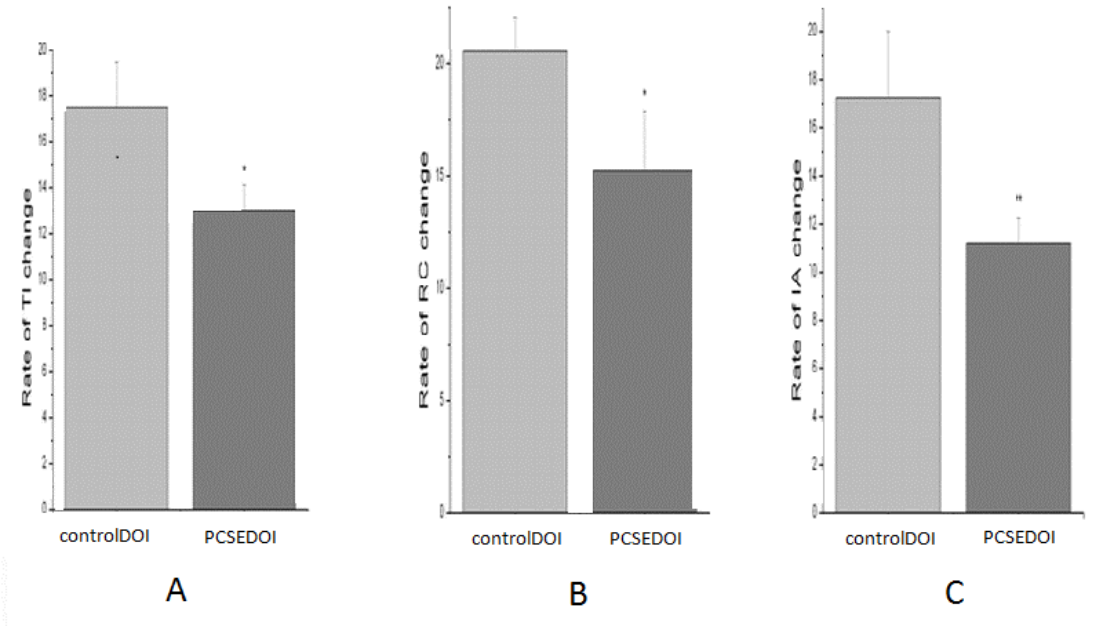


Fig. 3: Change rate comparison of DOI effect on RRDA of control group and PCSE group. (A) The rate inspiratory time of RRDA changes in two groups after application of DOI (40 μmol/L). (B) The rate respiratory cycle of RRDA changes in two groups after sustained DOI (40 μmol/L) applications. (C) The rate integral amplitude of RRDA changes in two groups after sustained DOI (40 μmol/L) applications. All values are mean values ± S.E.M. * *P*<0.05 compared to control group, ** *P*<0.01 compared to control group.

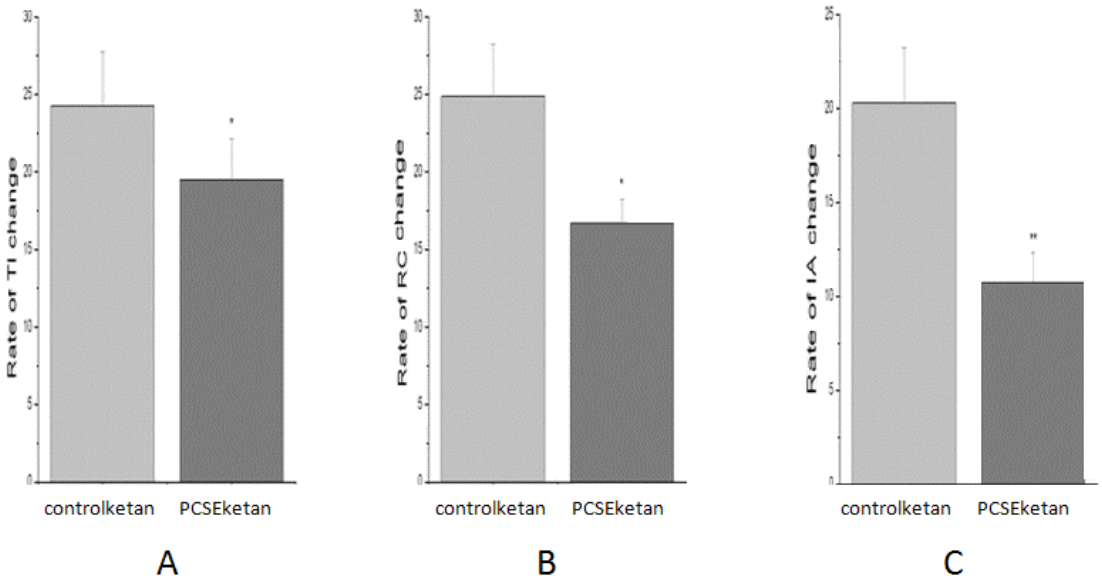


Fig. 4: Change rate comparison of Ketanserine effect on RRDA of control group and PCSE group.

(A) The rate inspiratory time of RRDA changes in two groups after application of Ketanserine (40μmol/L). (B) The rate respiratory cycle of RRDA changes in two groups after sustained Ketanserine (40μmol/L) applications. (C) The rate integral amplitude of RRDA changes in two groups after sustained Ketanserine (40μmol/L) applications. All values are mean values ± S.E.M. * P<0.05 compared to control group, ** P<0.01 compared to control group.

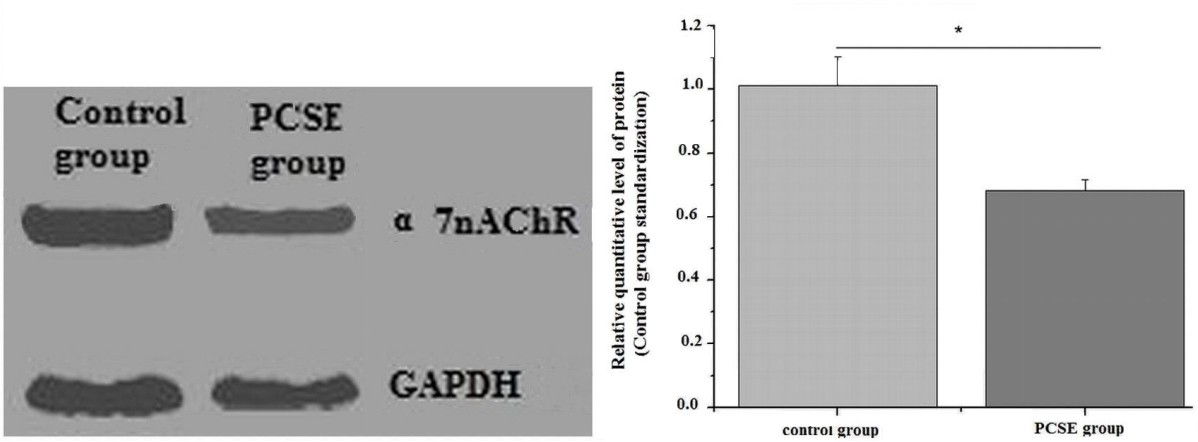
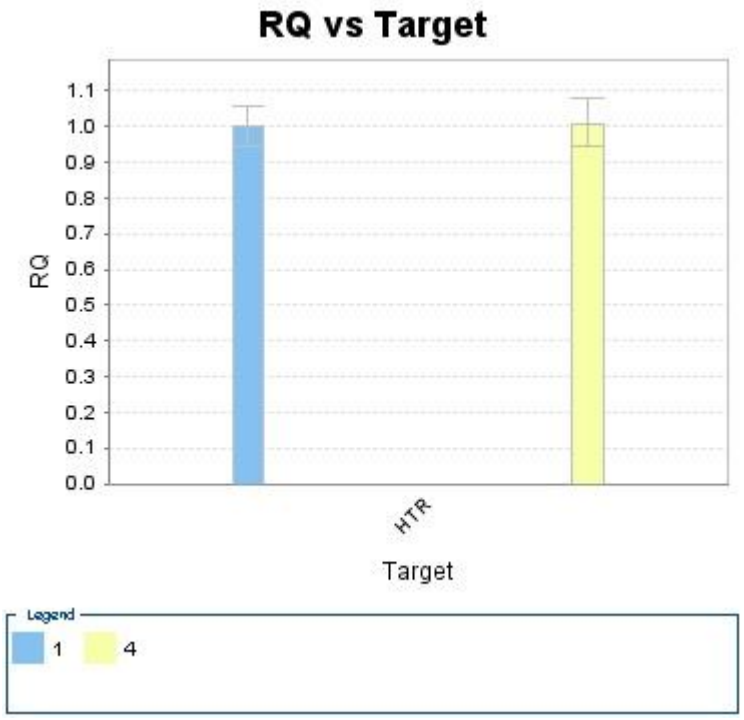


Fig. 5: PCSE down-regulated 5-HT2AR in PBC.



(1-control 4-PCSE)

Fig. 6: Real time quantitative RT-PCR measurements of 5-HT_{2A} receptor mRNA.