Repeated formaldehyde inhalation impaired olfactory function and changed SNAP25 proteins in olfactory bulb

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Background: Formaldehyde inhalation exposure, which can occur through occupational exposure, can lead to sensory irritation, neurotoxicity, mood disorders, and learning and memory impairment. However, its influence on olfactory function is unclear.

Objectives: To investigate the mechanism and the effect of repeated formaldehyde inhalation exposure on olfactory function.

Methods: Rats were treated with formaldehyde inhalation (13.5 ± 1.5 ppm, twice 30 minutes/day) for 14 days. Buried food pellet and locomotive activity tests were used to detect olfactory function and locomotion. Western blots were used to evaluate synaptosomal-associated protein 25 (SNAP25) protein levels in the olfactory bulb (OB) lysate and synaptosome, as well as mature and immature olfactory sensory neuron markers, olfactory marker protein (OMP), and Tuj-1. Real-time polymerase chain reaction (PCR) was used to detect SNAP25 mRNA amounts.

Results: Repeated formaldehyde inhalation exposure impaired olfactory function, whereas locomotive activities were unaffected. SNAP25 protein decreased significantly in the OB, but not in the occipital lobe. SNAP25 also decreased in the OB synaptosome when synaptophysin did not change after formaldehyde treatment. mRNA levels of SNAP25A and SNAP25B were unaffected. Mature and immature olfactory sensory neuron marker, OMP, and Tuj-1, did not change after formaldehyde treatment.

Conclusion: Repeated formaldehyde exposure impaired olfactory function by disturbing SNAP25 protein in the OB.

Keywords: Formaldehyde inhalation, SNAP25, Olfactory function, Synaptosome

Introduction

Formaldehyde is widely used in multiple industries and can be found in substances such as lubricants, adhesives, fertilizers, germicides, dyes, and disinfectants. It is toxic to mammals, resulting in mutagenicity, genotoxicity, sensory irritation, neurotoxicity, carcinogenicity, and learning and memory impairment.^{1,2} It has been previously established that low-level formaldehyde exposure can influence odor sensitivity and long-term formaldehyde inhalation can change cellular morphologies in the olfactory bulb (OB).^{3,4} However, less is known about whether olfactory function is negatively affected by long-term formaldehyde inhalation exposure, which can occur from being employed in a pathology department or living in a newly finished or painted house.

Formaldehyde exposure influences neurotransmitters, synapse-related proteins, and gene levels.^{2,5,6}

Formaldehyde inhalation impairs learning and memory through changing SNARE (soluble *N*-ethylmaleimide-sensitive factor attachment protein) proteins, including synaptosomal-associated protein 25 (SNAP25).¹ SNAP23, a homolog of SNAP25, was found to decrease in the plasma of rats after formaldehyde inhalation.⁷ However, there are no studies that examine how SNAP25 changes in the OB after formaldehyde inhalation.

The aim of this study was to investigate the effects of repeated formaldehyde inhalation on olfactory function and to explore the mechanism detecting protein and mRNA levels of SNAP25, which contributes to neurotransmission, in the OB lysate and synaptosome.

Methods

Animals

Male Sprague–Dawley rats ranging from 160–180 g were obtained from the Institute of Animal care of Jilin University (Changchun, China). They were

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group-housed in a standard animal care room with a temperature of $22 \pm 1^\circ\text{C}$, a humidity of $50 \pm 5\%$, and 12-hour light and dark cycle. The rats were allowed to acclimatize to the environment and were provided with food and water *ad libitum* except when placed in the exposure chambers or the behavioral testing apparatus. The rats were divided into control and formaldehyde treatment groups, with eight rats per group.

Formaldehyde inhalation exposure

The formaldehyde exposure process has been described previously.² In summary, rats were exposed to evaporated formaldehyde (13.5 ± 1.5 ppm) (treatment group) or fresh air (control group, two times a day, with each exposure lasting 30 minutes (7:00 a.m.–7:30 a.m. and 19:00 p.m.–19:30 p.m.) in a static toxification chamber ($54 \times 31 \times 34$ cm). After each exposure, the chambers were cleaned and dried in preparation for future treatments. All experiments were approved by the animal care committee of Jilin University and performed in accordance with the animal care guidelines of the Chinese Council.

Buried food pellet and locomotive activity test

A modification of the buried food pellet test was used to assess olfactory function.⁸ After formaldehyde treatment, animals were food restricted overnight and trained to locate, dig up, and eat a 0.5–1 g piece of rodent chow. The food was buried approximately 1 cm under the 7.5 cm rat bedding material. The position of the pellet was randomly changed daily. Training and testing was conducted in a $54 \times 31 \times 34$ cm closed non-transparent chamber.

Food pellet latency was defined as the time between placing the rat into the cage and when it uncovered the food pellet and grasped it in its forepaws and/or teeth. Animals consumed the pellet and were returned to their cage. If the rat did not find the food pellet within 10 minutes, it was returned to its home cage and the time was recorded as 600 seconds. The bedding in the test chamber was disarranged between trials.

Brain lysate and synaptosome preparation

Rats were decapitated after being anesthetized with an intraperitoneal injection of chloral hydrate (350 mg/kg). The OB lysate, synaptosome, and occipital lobe lysate were prepared as previously described.²

Real-time polymerase chain reaction (PCR) and Western blots analysis

Total RNA in the OB was extracted with Trizol, and reverse transcription was performed according to the datasheet of PrimeScriptTM RT-PCR Kit (Takara, Dalian, China). The primers were: **SNAP25A** (forward, 5'-TGC TGT GGC CTT TTC ATA-3'; reverse, 5'-TCT GGC GAT TCT GGG TG-3'),

SNAP25B (forward, 5'-AAC TGG AAC GCA TTG AGG A-3'; reverse, 5'-TCT GGC GAT TCT GGG TG-3'), and **GAPDH** (forward, 5'-AGA CAG CCG CAT CTT CTT GT-3'; reverse, 5'-CTT GCC GTG GGT AGA GTC AT-3'). The real-time PCR reaction system was performed in a 20 μl reaction containing: $2 \times$ Master Mix (10 μl), ROX Reference Dye II ($50 \times$) 0.4 μl , Primer F/R (0.4 μl each, 10 $\mu\text{mol/l}$), sample cDNA (1 μl), and MilliQ H₂O (7.8 μl). The amplification conditions were 95°C for 15 seconds, 40 cycles of 95°C for 5 seconds, and 60°C for 34 seconds. The relative expression of the ESR gene was calculated from the cycle threshold (C_t) value using the dd C_t method for quantification.

Using the prepared brain lysate and synaptosome fraction, protein quantity was determined by BCA kit (Pierce Biotechnology Inc., Rockford, IL, USA). Protein samples were separated by 12% sodium dodecyl sulphate–polyacrylamide gel electrophoresis and electro-transferred to a polyvinylidene difluoride membrane (Millipore, Billerica, MA, USA). The membrane was blocked in 5% skim milk in TBST (0.075%) for 1 hour at room temperature and then incubated in primary antibodies in TBST (0.075%) at 4°C overnight. The antibodies dilutions were 1:4000 for SNAP25 (BD Transduction), 1:1000 for Tuj-1 (Santa Cruz Biotechnology, Santa Cruz, CA, USA), and 1:2000 for olfactory marker protein (OMP) (Wako, Richmond, VA, USA). After washing three times in TBST for 10 minutes, the membrane was incubated in horseradish peroxidase-labeled second antibody for 2 hours at room temperature. The membrane was then washed and detected by chemiluminescence (Pierce Biotechnology Inc.).

Data analysis

All data were expressed as group means $\pm$ standard error of the mean (SEM). Data from the buried food pellet, locomotive activity, real-time PCR, and Western blots analyses were evaluated by unpaired *t*-test. A statistical significant difference was set at $P < 0.05$.

Results

Olfactory function decreased after repeated formaldehyde inhalation exposure

Buried food pellet tests were performed after exposing rats to formaldehyde inhalation treatment to evaluate the effects on olfactory function. One of six tests resulted in a significant increase in the latency time to find food in the treatment group (Fig. 1A). All rats performed similarly on locomotive activity test that followed (Fig. 1B).

SNAP25 changes after repeated formaldehyde inhalation

After formaldehyde inhalation treatment, SNAP25 in the OB lysate and synaptosome decreased significantly

Figure 1 Repeated formaldehyde inhalation exposure impaired olfactory function of rats. (A) Olfactory function evaluated by buried food pellet test. (B–E) Locomotive activity. $N=8$. Error bar means SEM.

compared to the control group, while SNAP25 in the occipital lobe did not change after formaldehyde treatment (Fig. 2A). Further real-time PCR showed that mRNA levels of SNAP25A and SNAP25B were similar in the formaldehyde-treated and control rats (Fig. 2B).

Olfactory sensory neuron maturity were unaffected after formaldehyde inhalation exposure

Western blots of mature and immature olfactory sensory neuron markers, OMP and Tuj-1, were

similar in the formaldehyde treated and control rats (Fig. 3).

Discussion

We found that repeated formaldehyde inhalation exposure influenced olfactory function, while mature or immature olfactory sensory neurons in the OB were unaffected. SNAP25 protein, but not mRNA, decreased in the OB lysate and synaptosome. These results suggest that a decrease of SNAP25 protein in the OB and its synaptosomal fraction may contribute

Figure 2 SNAP25 proteins in the OB lysate and synaptosome, decreased after formaldehyde inhalation, while mRNA was unaffected. (A and B) Western blots of SNAP25 protein in the OB and OL lysate and statistical analysis. (C and D) Western blots of synaptophysin and SNAP25 in the OB synaptosome and statistical analysis. (E and F) Real-time PCR of mRNA of SNAP25A and SNAP25B in the OB. Ct, control; OB, olfactory bulb; OL, occipital lobe. $N=5$. * $P<0.05$. Error bar means SEM.

to olfactory function impairment after repeated formaldehyde inhalation exposure.

Formaldehyde exposure has been found to influence neurotransmitters, synapse-related proteins, and gene levels.^{2,5,6} Formaldehyde inhalation also decreases SNAP25 in the hippocampus and disturbs triiodothyronine (T3), thyroxine (T4) thyroid-stimulating hormone, adrenocorticotropin, and corticosterone.^{1,9} A relationship between hypothyroidism and reduced mRNA and protein levels of SNAP25 has been reported in neonatal brains.¹⁰ Additionally and in line with our results, a proteomic study revealed that SNAP23, a homolog of SNAP25, decreased significantly in rat plasma after formaldehyde inhalation.¹¹ These findings support our hypothesis that SNAP25 (or its homologs) are sensitive to formaldehyde exposure.

A decrease in SNAP25 is a predictor of neuronal loss or impaired synaptogenesis.¹² The targeted deletion of SNAP25 in mice has been found to result not only in neurotransmission impairment, but also embryonic or prenatal death.¹³ There were preferential

disturbances of SNAP25 and its homolog protein in neurodegeneration and neuroendocrine disturbances.¹⁴ SNAP25, a component in the SNARE complex, decreased in the hippocampus after formaldehyde inhalation, which may be responsible for learning and memory impairment.¹⁵ We hypothesize that SNAP25 changes may lead to olfactory function impairment as a result of repeated formaldehyde inhalation exposure. This study contributes to the understanding of brain damage caused by formaldehyde inhalation exposure.

Disclaimer Statements

Contributors Qi Zhang is the major performer of current study. She constructed animal models with repeated formaldehyde treatment. She collected samples, isolated synaptosome, and ran Western blots. Weiqun Yan helped Qi to construct animal models, and he also help to run RT-PCR experiments. Yang Bai taught Qi and Yan about anatomical locations of those brain samples. Yingqiao Zhu helped to did statistical

Figure 3 Formaldehyde inhalation didn't change olfactory sensory neuron maturation. (A) Western blots of OMP and Tuj-1. (B and C) Quantification analysis. Ct, control. $N=3$. Error bar means SEM.

analysis, and gave suggestive advice on design of this study. Jie Ma provided the whole idea of current design, and helped to make next plan, which made this study.

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Conflicts of interest The authors report no conflicts of interest.

Ethics approval There is no patient involved in this study. For those animals, all experiments were conducted following an approved protocol from the animal care committee of Jilin University and performed in accordance with the animal care guidelines of the Chinese Council.

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