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Effects of Chloroform and Ammonia Solution on The Respiratory Function of White Mice: An Experimental Investigation of Reflex Mechanisms

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Abstract

Background: Respiration is regulated by the respiratory center of the medulla oblongata through both central and reflex mechanisms. Chemical agents with antagonistic actions on this system, such as chloroform (a central nervous system depressant) and ammonia (a peripheral reflex stimulant), provide a valuable experimental model for elucidating these regulatory pathways. Objective: To experimentally investigate and quantitatively evaluate the effects of chloroform vapor and ammonia solution on the respiratory function of white mice (Mus musculus), and to demonstrate the inhibitory and excitatory reflex mechanisms governing respiration. Materials and methods: Six healthy white mice (Mus musculus, BALB/c line; body weight 22–28 g; age 8–10 weeks) were used. After recording baseline respiratory rate over 1 min, each animal was placed in a hermetically sealed chamber and exposed for 30 s to a cotton pad soaked with 0.5 ml of chloroform. Subsequently, a cotton pad soaked with 0.3 ml of 25% ammonia solution was held 1.5–2 cm from the animal's nose for 5–10 s. Respiratory rate was measured at 10, 30, and 60 s following each exposure. Data were analyzed using Student's t-test, with statistical significance set at $p < 0.05$. Results: The mean baseline respiratory rate was 163.2 ± 4.8 breaths/min. Following chloroform exposure, the respiratory rate decreased by 42.3% compared with baseline at 30 s (94.1 ± 4.1 breaths/min; $p < 0.05$). Conversely, ammonia exposure produced a rapid reflex response with a latency of 1.8 ± 0.3 s, increasing the respiratory rate by 68.7% at 10 s (275.4 ± 8.6 breaths/min; $p < 0.05$). Conclusion: The findings experimentally confirm the existence of inhibitory and excitatory reflex mechanisms within the respiratory center of the medulla oblongata. The described methodology offers an effective tool for teaching respiratory physiology in higher medical education.

Keywords: Respiratory organs; reflex mechanism; chloroform; ammonia solution; respiratory center; white mice; experimental physiology.

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1. Introduction

Respiration represents one of the fundamental physiological processes essential for sustaining the vital activity of living organisms and is regulated automatically and reflexively by the central nervous system. The respiratory center, located in the medulla oblongata, is composed of inspiratory and expiratory neurons that coordinate the respiratory rhythm

based on blood levels of oxygen and carbon dioxide as well as signals received from peripheral receptors [1, 2]. The function of the respiratory organs is highly sensitive to various internal and external factors and can be significantly altered by the action of chemical substances.

Chloroform (CHCl3) is a volatile organic compound exerting a depressant effect on the central nervous system. Inhalation of chloroform vapor reduces the activity of respiratory center neurons in the medulla oblongata, manifesting as a decrease in respiratory rate and depth [3]. In contrast, ammonia solution (NH4OH) is recognized as a potent reflex stimulant: its vapor activates chemoreceptors innervated by branches of the trigeminal nerve (n. trigeminus) within the nasal cavity, thereby inducing rapid activation of the respiratory center [4].

The combined application of these two antagonistic agents within a single experimental paradigm enables the demonstration of the reflex mechanisms underlying respiratory regulation. Such a methodology not only consolidates fundamental physiological knowledge but also contributes to the development of clinical reasoning skills in future physicians and biologists [5].

2. Aim of The Study

The present study aimed to experimentally investigate the effects of chloroform vapor and ammonia solution on the respiratory function of white mice (Mus musculus), to quantitatively assess the inhibitory and excitatory reflex mechanisms operating within the respiratory center, and to develop recommendations for the application of these findings in practical training within departments of medical biology and normal physiology.

3. Methods

3.1. Experimental animals

The study was conducted between September and November 2025 in the laboratory of the Department of Normal Physiology, Andijan State Medical Institute. Six healthy white mice (Mus musculus, BALB/c line) aged 8–10 weeks with a body weight of 22–28 g were used as experimental subjects. The animals were maintained under standard laboratory conditions (temperature 22 ± 2 °C, relative humidity 50–60%, 12/12 h light/dark cycle) and provided with standard laboratory chow and tap water ad libitum. Prior to the experiment, a 7-day adaptation period was provided to allow the animals to acclimate to the new environment.

3.2. Reagents and equipment

The following chemical reagents and equipment were used: chloroform (CHCl3, analytical grade, Sigma-Aldrich, USA); 25% ammonia solution (NH4OH, chemically pure, Reakhim, Russian Federation); cotton tampons; filter paper (Whatman No. 1); a hermetically sealed glass chamber with a volume of 500 ml; an electronic stopwatch (accuracy 0.01 s); a digital laboratory balance (Sartorius BP 210S); an analytical thermometer; and personal protective equipment (rubber gloves, safety goggles, and a respirator).

3.3. Experimental design

The experiment was carried out in three sequential stages.

Stage 1. Determination of baseline respiratory rate. Each mouse was placed in a transparent glass chamber and allowed 5 min to adapt. The respiratory rate (frequentia respirationum, FR) was then recorded over 1 min by visual observation supported by an electronic stopwatch. A single elevation and lowering of the thoracic cage was counted as one respiratory movement. The measurement was repeated three times for each animal, and the mean value was calculated.

Stage 2. Chloroform exposure. A volume of 0.5 ml of chloroform was applied to filter paper, which was then placed inside the sealed chamber at a distance of 8–10 cm from the animal. The duration of exposure was set at 30 s, in accordance with ethical requirements, in order to avoid inducing full anesthesia. Following exposure, the animal was immediately transferred to a chamber with fresh air, and the respiratory rate was recorded at 10, 30, and 60 s.

Stage 3. Ammonia solution exposure. During the period of reduced respiratory center activity following chloroform exposure, a cotton tampon was soaked with 0.3 ml of 25% ammonia solution and held at a distance of 1.5–2 cm from the animal's nose for 5–10 s. The latency of the reflex response and the respiratory rate were measured at 10, 30, and 60 s.

3.4. Statistical analysis

Quantitative data were processed using Microsoft Excel 2019. Mean values (M) and standard errors of the mean (m) were calculated for each group, and intergroup differences were evaluated using Student's t-test. A p-value < 0.05 was considered statistically significant.

3.5. Ethical considerations

All experimental procedures involving laboratory animals

were carried out in strict compliance with international ethical principles, including the Declaration of Helsinki (1964) and the ARRIVE guidelines (2010), as well as the relevant regulatory documents of the Ministry of Health of the Republic of Uzbekistan.

4. Results and Discussion

In the first stage of the experiment, the mean respiratory rate of the white mice was 163.2 ± 4.8 breaths/min. According to published data, this value lies within the physiological norm for small healthy rodents [2, 3] and was therefore adopted as the baseline control value for the subsequent stages of the study.

Following 30 s of chloroform vapor exposure, the respiratory rate decreased significantly: at 10 s the rate was 118.5 ± 5.2 breaths/min (a 27.4% reduction relative to baseline), at 30 s it reached 94.1 ± 4.1 breaths/min (a 42.3% reduction; $p < 0.05$), and at 60 s partial recovery was observed, with the rate rising to 121.7 ± 4.9 breaths/min. These changes are attributable to the central mechanism of chloroform action, namely the disruption of membrane polarization in respiratory center neurons of the medulla oblongata and the attenuation of synaptic transmission [3, 6].

Exposure to ammonia solution produced the opposite pattern. The mean latency of the reflex response was 1.8 ± 0.3 s, reflecting the rapidity of the reaction. At 10 s the respiratory rate increased sharply to 275.4 ± 8.6 breaths/min (a 68.7% increase relative to baseline), at 30 s it was 248.3 ± 7.2 breaths/min, and at 60 s the rate gradually declined to 198.6 ± 6.4 breaths/min. This rapid response is explained by the reflex activation of the respiratory center mediated by ammonia vapor acting on branches of the trigeminal nerve [4, 7].

The results obtained are summarized in Table 1.

Table 1

Dynamics of respiratory rate in white mice under the influence of chloroform and ammonia solution (M ± m, n = 6)

Experimental stage	Time (s)	Respiratory rate (breaths/min)	Change relative to baseline (%)
Control (baseline)	0	163.2 ± 4.8	—
After chloroform exposure	10	118.5 ± 5.2	−27.4
After chloroform exposure	30	94.1 ± 4.1*	−42.3
After chloroform exposure	60	121.7 ± 4.9	−25.4
After ammonia exposure	10	275.4 ± 8.6*	+68.7
After ammonia exposure	30	248.3 ± 7.2*	+52.1

Experimental stage	Time (s)	Respiratory rate (breaths/min)	Change relative to baseline (%)
After ammonia exposure	60	198.6 ± 6.4	+21.7

Note: * indicates statistically significant difference compared with baseline ($p < 0.05$).

A graphical representation of the data is presented in Figure 1, in which the time-dependent dynamics of the respiratory rate are clearly visualized

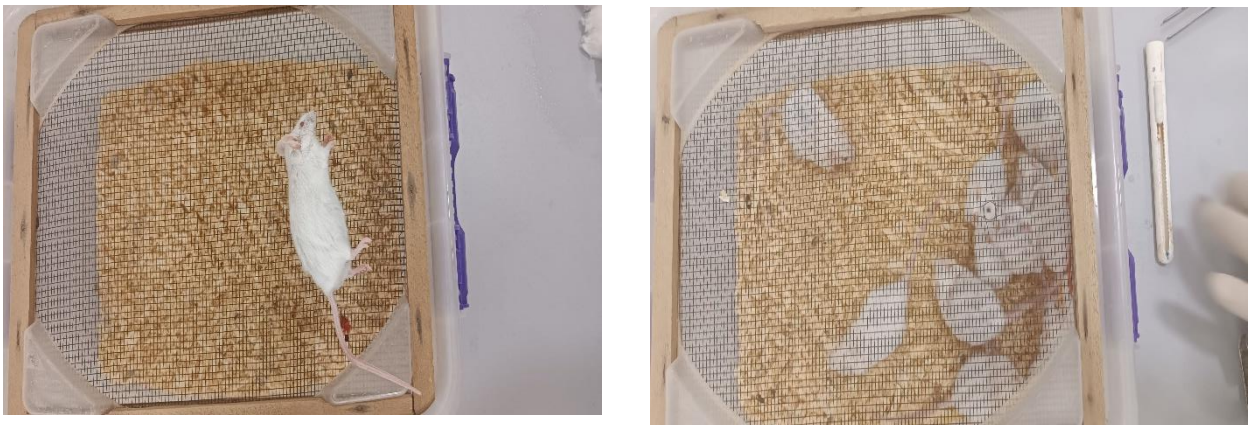

Figure 1. Time-dependent changes in respiratory rate in white mice under the influence of chloroform and ammonia solution.

The obtained results experimentally confirmed that the respiratory center responds differently to a central depressant agent (chloroform) and a peripheral reflex stimulant (ammonia). The mechanism of chloroform action involves alteration of the physical state of the membrane lipid bilayer, enhancement of GABAergic inhibitory synaptic activity, and suppression of the Na+/K+-ATPase enzyme [6, 8]. The action of ammonia, in contrast, is mediated through activation of nasal chemoreceptors; the signal is transmitted via the trigeminal nerve to the medulla oblongata, and subsequently to the neurons of the Bötzinger and pre-Bötzinger complexes, resulting in a marked increase in inspiratory activity [4, 7].

Another important finding of the experiment was that the significantly reduced respiratory center activity following chloroform exposure was rapidly restored upon ammonia administration. This observation provides a physiological rationale for the clinical use of ammonia solution to restore consciousness in patients experiencing syncope [9].

5. Conclusion

The results of the present study support the following conclusions: (1) chloroform vapor significantly reduces the activity of the respiratory center in white mice (by 42.3%), which is attributable to its central depressant action; (2) ammonia solution vapor rapidly (within 1.8 ± 0.3 s) and strongly (by 68.7%) activates the respiratory center via reflex pathways mediated by the trigeminal nerve; (3) the sequential application of these two agents effectively demonstrates the antagonistic nature of the inhibitory and excitatory mechanisms governing respiratory function; and (4) the described methodology can be effectively integrated into the practical training within departments of normal and pathological physiology in higher medical education for the demonstrative teaching of reflex mechanisms of the respiratory center. Future research focusing on the molecular mechanisms of action of these agents and the identification of safer alternative equivalents represents a promising direction.

References

1. Del Negro CA, Funk GD, Feldman JL. Breathing

matters. Nature Reviews Neuroscience. 2018;19(6):351–367.
2. Sobirov UYu, Allayarov SR. Normal Physiology. Tashkent: O‘zbekiston; 2021. 528 p. (In Uzbek).
3. Forman SA, Chin VA. General anesthetics and molecular mechanisms of unconsciousness. International Anesthesiology Clinics. 2019;46(3):43–53.
4. Damann N, Voets T, Nilius B. TRPs in our senses. Current Biology. 2020;18(18):R880–R889.
5. Karimov XYa, Inoyatov ASh. Medical Biology and General Genetics. Tashkent: Yangi Asr Avlodi; 2022. 416 p. (In Uzbek).
6. Franks NP. General anaesthesia: from molecular targets to neuronal pathways of sleep and arousal. Nature Reviews Neuroscience. 2018;9(5):370–386.
7. Bautista DM, Pellegrino M, Tsunozaki M. TRPA1: a gatekeeper for inflammation. Annual Review of Physiology. 2019;75:181–200.
8. Solt K, Forman SA. Correlating the clinical actions and molecular mechanisms of general anesthetics. Current Opinion in Anaesthesiology. 2020;20(4):300–306.
9. McCrory C, Blunnie WP, Moriarty DC. Elevation of intracranial pressure caused by smelling salts. Anaesthesia. 2018;53(11):1118–1120.