Effects of methyl methacrylate on the excitability of the area postrema neurons in rats

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Abstract

Objectives

The aim of the present study is to demonstrate the effects of inhaled methyl methacrylate (MMA) on the excitability of neurons in the area postrema (AP). We also investigated the relation between vagal afferent inputs and responding cells in the AP.

Methods

We set up two groups of experimental animals, such as rats inhaling MMA and rats inhaling room air. To visualize the changes of AP neuron excitability after inhalation of MMA for 90 min, c-Fos protein expression was identified and quantified by immunohistochemical analysis. Some rats receiving ventral gastric branch vagotomy were also subjected to the abovementioned experiment.

Results

The number of c-Fos-immunoreactive (Fos-ir) cells in the MMA group was more than six times greater than that of the control group (statistically significant, $p<0.01$). In vagotomized rats inhaling MMA, markedly smaller number of Fos-ir cells was identified in the AP compared to that of rats inhaling MMA without vagotomy.

Conclusions

These results indicate that inhalation of MMA increases neuronal excitability in the AP,

suggesting that vagal afferent inputs are involved in the induction mechanism of Fos-ir cells by MMA.

Keywords: area postrema, methyl methacrylate, c-Fos, vagotomy

1. Introduction

The area postrema (AP) lacks the blood-brain barrier and is well known as the chemoreceptor trigger zone for emesis [1]. Previous studies have revealed the receptivity of the AP neurons to many chemical substances; however, the effects of methyl methacrylate (MMA) remained to be determined. Therefore, we hypothesized that MMA inhalation increases the excitability of AP neurons triggering nausea and/or emesis.

The MMA is a major component in some popular dental resin that is often used in dental medicine, such as dental composite resins, dentures, temporary crowns, etc. As the MMA vaporizes easily at room temperature, dentists or patients possibly inhale a small amount of MMA during dental treatment. Although safety has been established for small amounts of MMA inhalation, health hazards such as nausea and vomiting are a concern if inhaled in large quantities [2]. For example, toluene, an organic solvent, vaporizes easily and excites the AP neurons [3]. We also investigated the role of vagal afferent inputs in the effects of inhaled MMA on the excitability of AP neurons, because a previous study has reported that the vagal afferent inputs are involved in the induction mechanism of cisplatin-induced emesis [4].

The present study demonstrated the MMA-induced upregulation of the AP

neuronal excitability using immunohistochemical analysis of c-Fos expression and the possible role of abdominal vagal afferent inputs in the effects of MMA on AP neurons.

2. Materials and Methods

2.1. Subjects

The Hokkaido University Animal Use Committee approved this study. Adult male Wister or SD rats (MMA, 5 rats; control, 5 rats; vagotomy, 3 rats; 150–600 g body weight) were housed individually under a 12/12 h light/dark cycle (lights on at 7 AM; off at 7 PM). Experiments were performed during the light phase.

2.2 Ventral gastric branch vagotomy

Ventral gastric branch vagotomy was performed as reported previously [5]. Briefly, rats were anesthetized and incised in the middle of the abdomen. The stomach was gently manipulated to pick out the ventral gastric branch of the subdiaphragmatic vagus. The ventral branch of the vagal nerve was transected, and pyloroplasty was performed to prevent pyloric stenosis. The pyloric sphincter was incised longitudinally (0.5–0.7 mm), and the incised part was sutured with silk sutures. After 10–14 days of recovery, the rats were subjected to the MMA inhalation experiment.

2.3 Administration of MMA

A rat was moved from the breeding cage to a desiccator and was habituated for 4 h before starting each experiment (Fig. 1). The rats without vagotomy were divided into two experimental groups: one group breathes MMA for 90 min (n = 5), and the other group breathes room air (n = 5). MMA liquid (3 mL) was dropped and volatilized in the desiccator at room temperature (approximately 25±3°C). Three rats that underwent vagotomy inhaled the MMA in the same manner as above. Immediately after inhalation of MMA or room air for 90 min, animals were deeply anesthetized with urethane and then perfused with 4% paraformaldehyde (PFA). Brains were carefully removed from the skull and fixed in 4% PFA at 4°C overnight, and then transferred to a 30% sucrose/PBS solution at 4°C until the brains sank to the bottom.

2.4 Immunohistochemical analysis

Coronal sections including the AP (Fig. 2A) [6] were cut at a thickness of 50 µm in the freezing microtome (FX-801; Yamato, Saitama, Japan) and kept in wells containing PBS at 4°C. Each section was rinsed thrice in PBS for 5 min, and sections were immersed in 80% methanol containing 0.3% hydrogen peroxide (H_2O_2) for 1 h at

room temperature. After three rinses in PBS for 10 min and immersion in blocking buffer (1.5% normal goat serum and 0.4% Triton X-100 in PBS) for 1 h at room temperature, sections were incubated in primary rabbit anti-cFos antibody (sc-52; Santa Cruz, Dallas, United States) diluted 1:5000 in blocking buffer at room temperature overnight or at 4°C for 3–5 days. Each section was rinsed thrice in PBS for 10 min and incubated in biotinylated anti-rabbit secondary antibody (VECTASTAIN Elite ABC kit, Vector Laboratories, Burlingame, United States) for 1 h at room temperature. Sections were rinsed twice in PBS for 10 min and rinsed twice in TBS for 10 min and then immersed in TBS containing 0.01% DAB and 0.05% nickel ammonium sulfate for 10 min at room temperature. Each section was treated with 0.0006% H_2O_2 for 7 min, and then sections were rinsed in TBS for 5 min and in PBS for 10 min and mounted on glass slides and coverslipped with Entellan (Merck, Darmstadt, Germany). Microscopic images of each section were analyzed on a personal computer using the Cell Counter plugin of ImageJ (NIH, Bethesda, United States). The average value of Fos-ir cells was determined by counting the number of cells from three sections in each animal, which was used for analysis as a representative value.

2.5 Statistical analysis

Using MATLAB (Mathworks, Statistics and Machine Learning Toolbox, Natick, United States), the number of Fos-ir cells between each experimental group was statistically analyzed by Wilcoxon signed-rank tests. Data were represented by means ± SEM.

3. Results

3.1 c-Fos expression in the AP through MMA inhalation

As shown in Figure 2A and 2B, many Fos-ir cells were found in the AP of the MMA group compared to the control group. The number of Fos-ir cells in the MMA group (168 ± 46 cells, n = 5 rats) was more than six times greater than that in the control group (26 ± 3, n = 5, p = 0.0079, Wilcoxon signed-rank tests, Fig. 2C). This result indicates that MMA inhalation increases neuronal excitability in the AP.

3.2 Effects of vagotomy on the expression of c-Fos in the AP

In vagotomized rats (n = 3), the average number of Fos-ir cells in the AP was 37±1 (Fig. 3A, B). This value was markedly smaller than that of the MMA group (168 ± 46, n = 5). This result suggests that vagal afferent inputs are involved in MMA-induced c-Fos expression of the AP neurons.

4. Discussion

In the present study, we investigated the effects of MMA on the excitability of neurons in the AP. The major findings in the present study were (1) MMA inhalation induced c-Fos expression in the AP, and 2) ventral gastric branch vagotomy suppressed c-Fos expression in the AP. These results revealed the sensitivity of AP neurons to MMA, suggested that vagal afferent inputs are involved in MMA-induced upregulation of neuronal excitability in the AP.

The c-Fos protein is often used as a marker of neuronal activity [7-10], because its expression indicates the facilitation of neuronal activity associated with the depolarization of membrane potentials, reaching a peak after 1.5–2 h from excitation of the neuron [11]. Some studies have reported that administration of cisplatin, which is an anticancer drug that causes nausea and vomiting as a side effect, introduces expression of the c-Fos protein in the AP of rodents [12, 13]. Another study has examined that c-Fos protein expression is related with the intake of kaolin, which is a measurement of nausea [14].

Two possible pathways of the effects of MMA on AP neuronal excitability are (1) circulating MMA induced upregulation of the vagal afferent inputs to AP neurons,

and 2) AP neurons may directly respond to circulating MMA. There are vagal afferent nerves that show the direct projection to AP neurons [15] and the indirect projection to AP neurons via the nucleus tractus solitarius (NTS) [16].

The present study suggests that vagal afferent inputs are involved in the excitation of the AP neurons by the MMA. Whether the MMA in the blood directly excites the AP neurons remains unexplained. To answer this question, we will perform another experiment using electrophysiological methods [17-24].

Considering the functional role of AP in triggering nausea and/or emesis, animals may feel nauseous during exposure to MMA in the present study. A study on humans has reported that inhalation of MMA caused respiratory tract irritation, weakness, fever, dizziness, nausea, headache, and sleepiness [2]. An animal study using rats has reported that the main clinical signs of acute MMA inhalation were depression, ataxia, and excessive salivation [25]. Some previous studies have reported MMA toxicity to nasal mucosal epithelial cells in rats [26, 27]. Raje et al. have subjected rats to MMA inhalation (100 ppm) for up to 4 hours, and they have reported several symptoms such as interalveolar congestion/hemorrhage, pulmonary vasodilatation, and edema; however, no histopathologic change was seen in the brain [28]. When rats inhaled a relatively high concentration of MMA (3000 ppm), extensive cerebellar

congestion and hemorrhage occurred in the cerebellar peduncles [29]. Increase of Fos-ir cells in the AP after MMA inhalation may occur because of MMA toxicity; however, further work is required for its elucidation.

Some previous studies have reported that intestinal motor activities and smooth muscle tonus were reduced by MMA inhalation (116 ppm) [30, 31]. In the present study, MMA-induced gastrointestinal motility disorder possibly increased afferent vagal nerve activity, resulting in the increased number of Fos-ir cells in the AP.

5. Conclusion

The present study revealed that AP neurons could respond to the inhalation of extreme amounts of MMA. Increases in the vagal nerve activity by MMA are possibly involved in the upregulation of neuronal excitability in the AP.

Ethical statement

The Hokkaido University Animal Use Committee approved this study.

Conflicts of interest

The authors declare no competing financial interests.

CRediT authorship contribution statement

Tomohiko Yoshizawa: conceptualization, methodology, validation, formal analysis, investigation, data curation, writing (original draft), writing (review and editing), and visualization. **Makoto Funahashi:** conceptualization, methodology, investigation, resources, writing (original draft), writing (review and editing), visualization, and funding acquisition.

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Figure legends

Fig. 1. Experimental design

Immediately after administration of MMA or room air for 90 min in the desiccator, rats are fixed with 4% paraformaldehyde (PFA)

Fig. 2. Fos-ir cells in the AP after MMA inhalation

A, A schematic diagram of the coronal section of the AP. The AP is located at the bottom of the fourth ventricle and lacks the blood-brain barrier, called the chemoreceptor trigger zone

B, Example photomicrographs of a coronal section of the area postrema region. Each lower panel is an enlarged view of the part surrounded by a solid line in the upper panel.

A lot of Fos-ir cells (white arrows) were found in the AP of the MMA group rats, whereas few cells in the control group rats. Scale bar: 100 µm (upper) and 50 µm (lower)

C, The average number of Fos-ir cells in the MMA group is significantly larger than that in the control group. **: $p < 0.01$, Wilcoxon signed-rank tests

Fig. 3. Effects of vagotomy on the c-Fos expression of the AP neurons

A, An example photomicrograph of a coronal section of the area postrema region in vagotomized rats. Scale bar: 100 µm

B, The average number of Fos-ir cells in the vagotomy group ($n = 3$) is markedly smaller than that in the MMA group ($n = 5$, same value as shown in Fig. 2C)

Fig.4. Schematic illustration of the possible mechanism of the MMA effects on the AP neurons

The present study suggests that MMA absorbed into the blood from the lungs increases abdominal vagal nerve activity, and the excitability of the AP neurons receiving synaptic inputs from the vagus nerve increased (solid arrows). Further, MMA may directly affect the AP neurons; however, it remains to be determined (dotted arrow)

Fig. 1

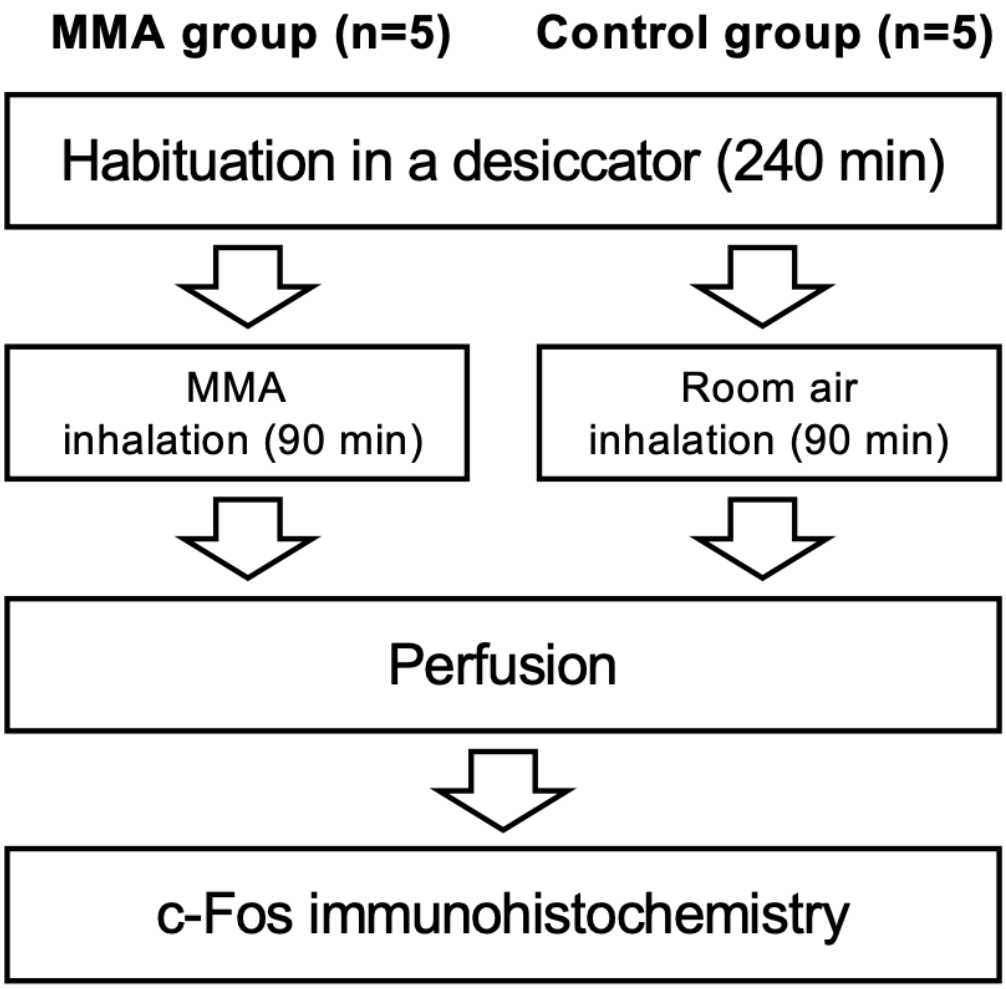

MMA group (n=5)
Control group (n=5)
Habituation in a desiccator (240 min)
MMA
inhalation (90 min)
Room air
inhalation (90 min)
Perfusion
c-Fos immunohistochemistry

Fig. 2

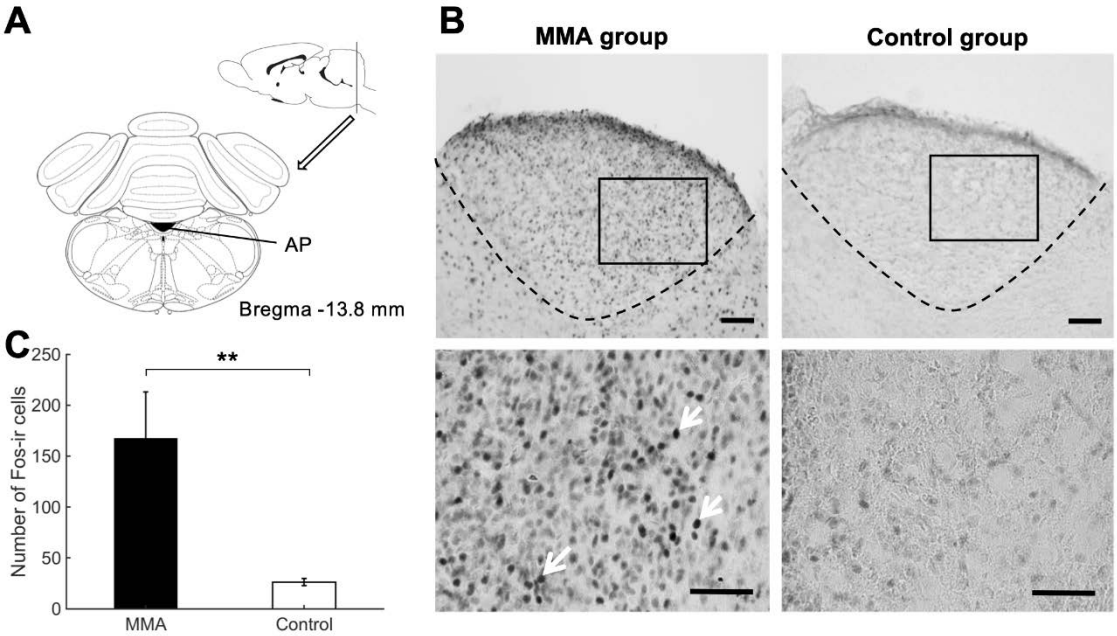

A
AP
Bregma -13.8 mm
B
MMA group
Control group
C
Number of Fos-ir cells
250
200
150
100
50
0
**
MMA
Control

Fig. 3

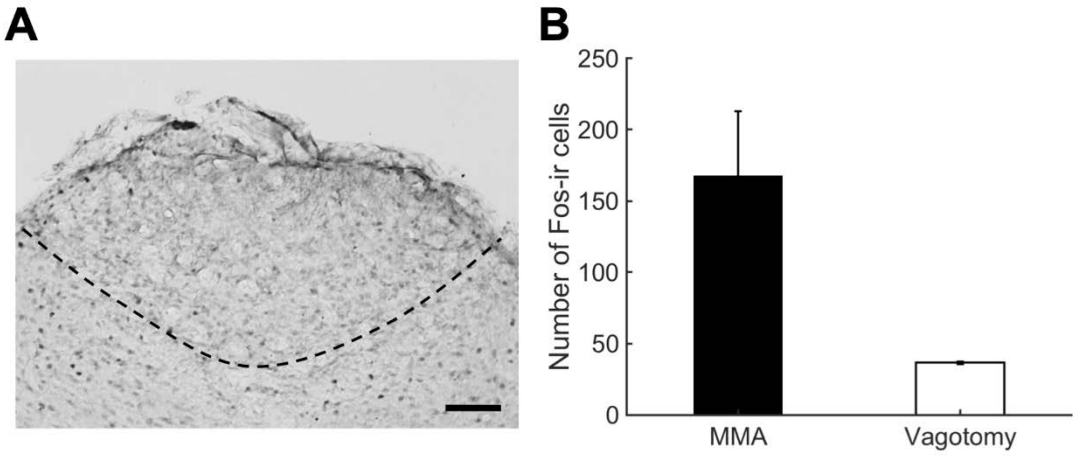
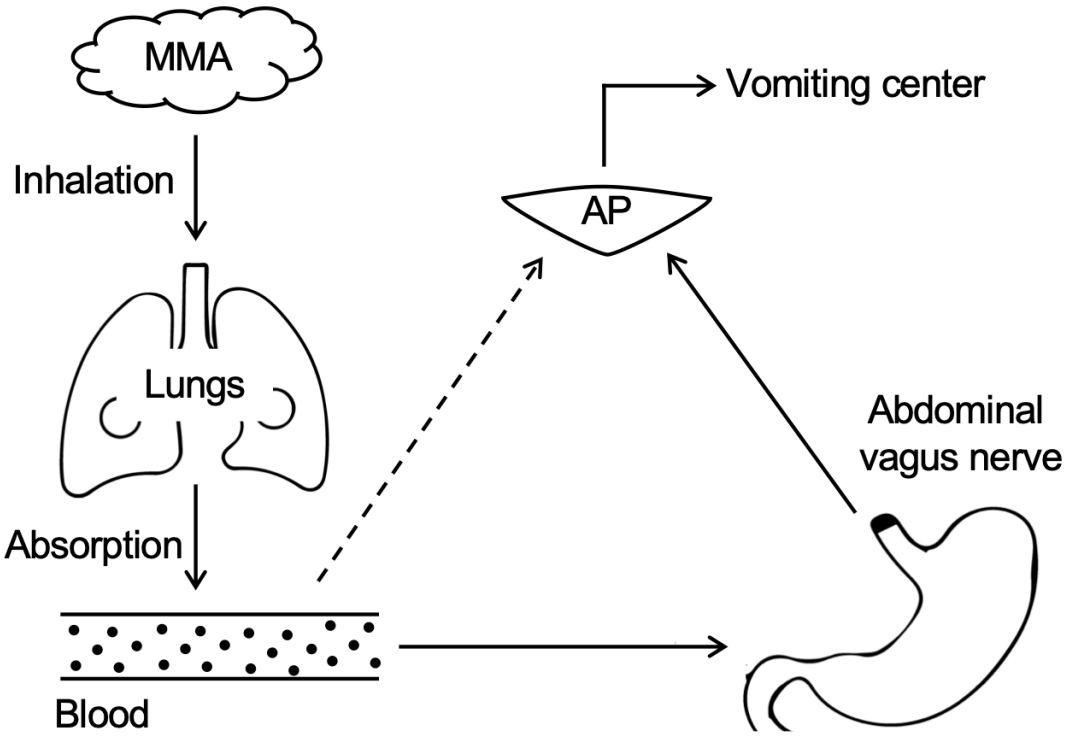


Fig. 4


MMA
Inhalation
Lungs
Absorption
Blood
AP
Vomiting center
Abdominal
vagus nerve