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SHORT COMMUNICATION

Isoflurane induces c-Fos expression in the area postrema of the rat

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Abstract

Introduction Volatile anesthetics are speculated to cause postoperative nausea and vomiting via stimulation of the chemoreceptor trigger zone (CTZ). However, the precise mechanism underlying the emetic action of these drugs is not well understood. In this study, we assessed whether isoflurane induced the expression of c-Fos, a neuronal activation marker, in the area postrema (AP), the locus of the CTZ, in rats, which do not have vomiting action.

Materials and methods Male rats were exposed to 1.3% isoflurane for 0–240 min, or to various concentrations of isoflurane (0, 1.3%, or 2.6%) for 120 min. Finally, the rats were exposed to 1.3% isoflurane for 120 min after ondansetron administration. After the treatments, immunohistochemistry of the rat AP was performed using c-Fos antibody staining.

Results One-way analysis of variance showed that isoflurane exposure significantly increased c-Fos expression in the AP; however, the rats pretreated with 4 mg/kg ondansetron showed significantly decreased c-Fos expression. Moreover, we evaluated the effect of the anesthetic on inducing pica in the rats, and found that kaolin intake was not influenced by isoflurane exposure.

Conclusion Overall, these results suggest that isoflurane activates AP neurons and may be involved in the emetic mechanism of isoflurane. This study further suggests the feasibility of using rats as a model for studying emetic mechanisms of drugs, despite their lack of vomit action.

Keywords Isoflurane · Area postrema · c-Fos · Ondansetron

Postoperative nausea and vomiting (PONV) remains a common complication of anesthesia. Approximately 25–30% of the patients experience PONV within the first 24 h of general anesthesia [1]. Among the various factors identified to be responsible for PONV [1, 2], volatile anesthetics are the most common cause via stimulation of the chemoreceptor trigger zone (CTZ) [1]. However, the precise mechanism underlying the emetic action of volatile anesthetics is not well understood. Some anti-emetic drugs are current used to manage PONV, including serotonin [5-hydroxytryptamine 3 (5-HT3)] receptor antagonists, dopamine receptor antagonists, corticosteroids, and neurokinin 1 receptor antagonists [3, 4]; however, the pathways involved in the ability of these drugs to suppress PONV are also poorly understood.

One of the major reasons for the slow progress in basic studies about PONV is that rodents, as typical animal models for assessing drug effects and mechanisms, do not have natural vomiting action, which is present in other small animals such as dogs and cats [5]. Previous studies in animals with vomiting actions demonstrated that emetics cause the expression of c-Fos, a neuronal activation marker, in the area postrema (AP), which is connected with vomiting behavior. The AP lies at the caudal end of the fourth ventricle, and was identified in the early 1950s as the locus of the CTZ responsible for emesis [6, 7]. The AP neurons are closely linked to the nucleus of the solitary tract (NST), and regarded as part of the emetic circuit [8]. In addition, recent studies in rats have shown that cisplatin and oxycodone, which trigger nausea and vomiting, induce the expression of c-Fos in the AP [9, 10].

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Therefore, we hypothesized that volatile anesthetics might also induce c-Fos in the rat AP. To test this possibility, we examined whether isoflurane at different concentrations and/or different times induced c-Fos expression in the rat AP. We further evaluated whether pretreatment of ondansetron, a 5-HT3 receptor antagonist, could inhibit this isoflurane-induced c-Fos expression.

All animal experiments were approved by the Committee for Animal Research of Hokkaido University Graduate School of Medicine (nos. 15-0016, 2015 and 19-0002, 2019). Ten-week-old male Wistar/ST rats were obtained from Japan SLC, Inc. (Shizuoka, Japan). The rats were handled in accordance with the Guidelines for the Care and Use of Laboratory Animals of Hokkaido University Graduate School of Medicine.

The study consisted of four experiments. In the first experiment, the rats were divided into the following five treatment groups according to the time of isoflurane exposure: 1.3% isoflurane with 40% oxygen (O_2) for 0 ($n=5$), 90 ($n=6$), 120 ($n=6$), 180 ($n=5$), or 240 ($n=6$) min. In the second experiment, the rats were divided into the following three treatment groups according to the concentration of the isoflurane gas: exposed to 0% ($n=5$), 1.3% ($n=5$), or 2.6% ($n=6$) isoflurane with 40% O_2 for 120 min. In the third experiment, the rats were divided into the following four treatment groups according to the preadministration dose of ondansetron: 0 ($n=5$), 1 ($n=6$), 2 ($n=5$), or 4 ($n=6$) mg/kg ondansetron injected intraperitoneally 30 min prior to exposure to 1.3% isoflurane with 40% O_2 for 120 min. For all treatments, the rats were placed in an acrylic chamber and exposed to a mixture of isoflurane and oxygen gas with air continuously delivered from an anesthesia machine (Excel 210 MRI compatible; Ohmeda) into the chamber through an inflow port. The concentrations of isoflurane and O_2 in the chamber were continuously monitored using an anesthetic gas monitor (M1026A Anesthetic Gas Module, Philips Medical System Corporation, Best, The Netherlands).

After the treatments, the animals were intraperitoneally injected with 100 mg/kg sodium pentobarbital, and perfused with 0.1 M phosphate-buffered saline (PBS) followed by 4% paraformaldehyde in 0.1 M phosphate buffer through the left ventricle into the ascending aorta. For AP extraction, the whole brain was stored in a solution of 20% sucrose in 0.1 M PBS, followed by incubation with 30% sucrose overnight at 4 °C each day. A portion of the brainstem was cut, 30-µm transverse sections at the AP level were cut at − 23 °C, and every third section was collected and placed on a glass slide.

The endogenous peroxidase activation of the tissues was quenched. After rinsing, the sections were incubated for 1 h in a blocking solution, and then incubated overnight with goat anti-c-Fos antibody (1:5000, catalog no. sc-52-G, Santa Cruz Biotechnology, Santa Cruz, CA, USA) in a blocking solution consisting of 1% normal donkey serum in PBS at 4 °C. The sections were then incubated for 1 h with biotinylated donkey anti-goat immunoglobulin (1:200; Vector Laboratories, Burlingame, CA, USA) in the same solution, followed by incubation for 1 h with avidin–biotin–peroxidase complex (Vector Laboratories) in PBS. The immunohistochemical reaction was visualized by incubation with 3,3′-diaminobenzidine (DAB) and nickel ammonium sulfate to which hydrogen peroxide was added (DAB kit, Vector Laboratories). After the staining procedure was completed, the sections were dehydrated and covered with coverslips for observation with bright-field microscopy (Olympus Model BX51TRF; Olympus Corporation; Japan) to identify c-Fos-positive cells by dense, black nuclear DAB staining. The three sections with the strongest staining intensities were photographed using a digital camera (Olympus Digital Camera, model U-LH100HGAPO, Olympus Optical; Japan), and the number of c-Fos-positive cells in the three AP sections was counted by an author blinded to the experimental group.

In the last experiment, we measured the consumption rate of kaolin after isoflurane exposure. Previous reports suggested that rodents craved non-food materials such as kaolin after emetic stimulation, in a phenomenon known as "pica" [11, 12]. In this study, kaolin intake was measured according to the method of Yamamoto et al. [11]. In brief, the feeding box was divided into two sections: standard feed was placed in one section, and kaolin pellets were placed in the other. Kaolin pellets were prepared as described by Takeda et al. [13]. After the rats were allowed to habituate to the experimental conditions for 1 week, 10-week-old male rats ($n=6$) were exposed to 2.6% isoflurane for 120 min. As a control, the rats ($n=6$) were placed in a box without anesthesia or food and water for 120 min. The kaolin intakes were measured before exposure and at 24 h after exposure. The intakes were weighed to the nearest 0.01 g.

All statistical analyses were performed using GraphPad Prism version 6 (GraphPad Software, Inc., San Diego, CA, USA). Data obtained in experiments 1–3 were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test, and data obtained in experiment 4 were analyzed using Student's *t* test. All averaged data are presented as the mean (standard deviation). Probability values (P) of $<5\%$ were considered statistically significant.

The number of c-Fos-positive cells in the AP significantly increased at 90–240 min after isoflurane exposure, reaching the peak level at around 120 min and 180 min (Fig. 1a). The number of c-Fos-positive cells in AP samples for the rats treated with isoflurane was significantly higher ($P<0.01$) than that of the control group (0% isoflurane; $P<0.01$) (Fig. 1b). The group intraperitoneally administered with 4 mg/kg ondansetron prior to isoflurane exposure showed a significant decrease ($P=0.02$) in the isoflurane-induced

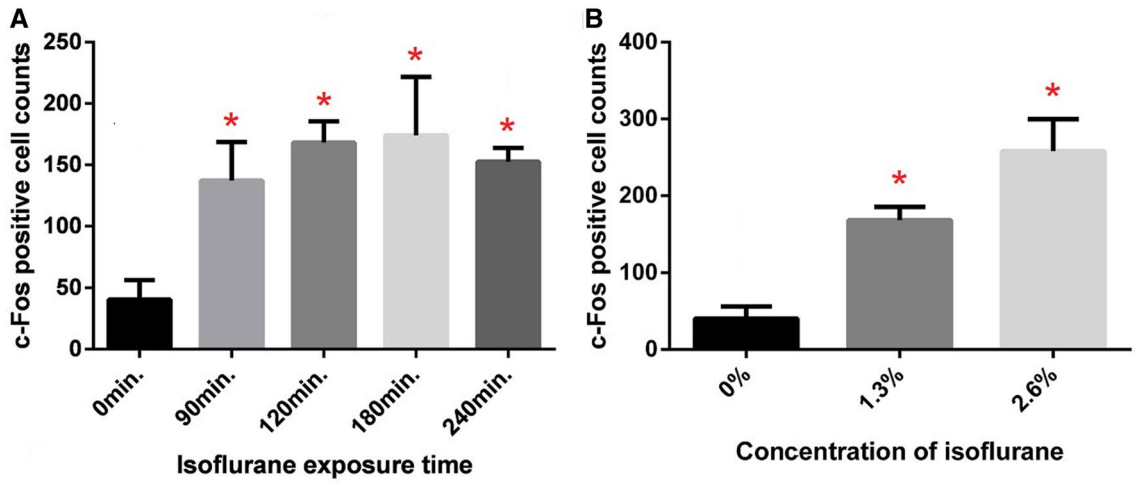

Fig. 1 **a** Number of c-Fos-positive cells in the AP of rats exposed to isoflurane for various time periods. *$P < 0.05$ vs. 0-min control group by Tukey's test. **b** Number of c-Fos-positive cells in the AP of rats exposed to various concentrations of isoflurane for 120 min. *$P < 0.05$ vs. 0% control group by Tukey's test

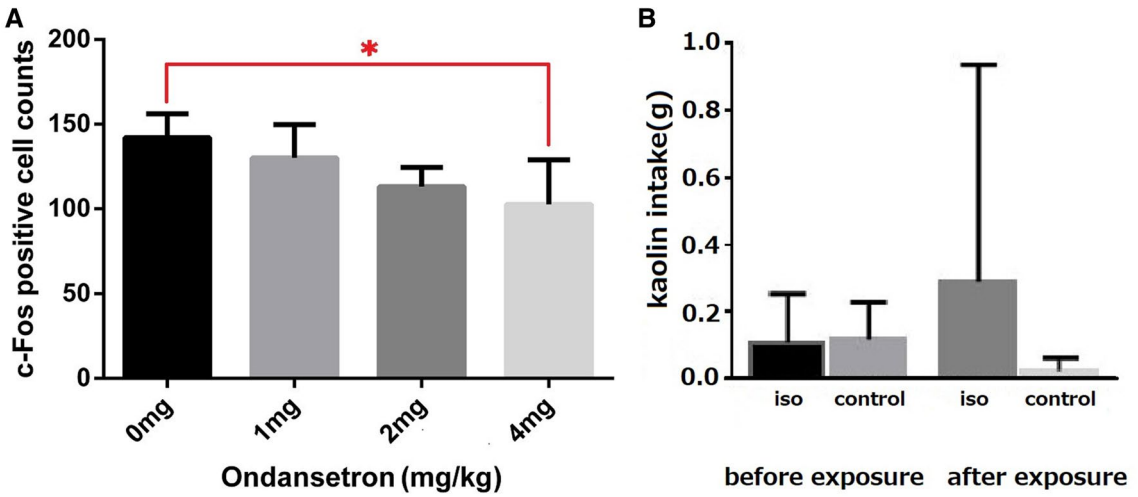

Fig. 2 **a** Number of c-Fos-positive cells in the AP in rats with preadministration of various concentrations of ondansetron prior to 1.3% isoflurane exposure for 120 min. *$P < 0.05$ vs. 0 mg control by Tukey's test. **b** Effect of isoflurane exposure on kaolin intake. There were no significant differences in kaolin intake between the control and isoflurane exposure groups before and at 24 h after exposure (Student's *t* tests)

increase in the number of c-Fos-positive cells in the AP (Fig. 2a). Only one rat was found to eat an abundant amount of kaolin; however, there was no significant difference in the kaolin intake before ($P = 0.89$) and at 24 h after exposure ($P = 0.34$) between the isoflurane exposure and control groups (Fig. 2b).

Overall, these results demonstrate that isoflurane exposure clearly and significantly increased c-Fos expression in the rat AP. c-Fos is a protein product of the immediate early gene, and is commonly used as a histologic marker of transsynaptic neuronal activation [14]. In animals with vomiting actions, emetics induce the expression of c-Fos in the AP, which is associated with the vomiting behavior. For example, examination of the c-Fos expression pattern in the brainstem and spinal cord in cats administered multiple emetic drugs (cisplatin, lobeline, protoveratrine, naloxone, and apomorphine) suggested that neurons involved in coordinating the emetic response might radiate from the AP and NST [15]. In another study, cisplatin administration caused emetic behavior in ferrets, resulting in c-Fos-like immunoreactivity in the AP and NST [16]. Similarly, cisplatin induced c-Fos expression in the AP in the house musk shrew (*Suncus murinus*) [17]. Although rodents do not have vomiting behavior, it is speculated that the fundamental structure and function of

the cerebrum are similar to those of animals with vomiting action. Indeed, cisplatin and oxycodone induce expression of c-Fos in the rat AP [9, 10], and we further confirmed herein that isoflurane could also induce c-Fos in the rat AP. To our knowledge, this is the first report that c-Fos expression is induced by a volatile anesthetic in the rat AP.

One study showed that halothane and isoflurane enhanced the function of 5-HT3 receptors [18]. These receptors are found peripherally on the abdominal vagal afferents and centrally in the AP and NST [19]. Gastrointestinal vagal afferent fibers play prominent roles in the first-line response of nausea and vomiting [20]. Indeed, vagotomy was shown to reduce the cisplatin-induced c-Fos expression to some degree in the rat NST [21]. In addition, volatile anesthetics (halothane, isoflurane, and sevoflurane) could inhibit the respiratory response via vagal C-fiber afferents in dogs [22]. By contrast, a recent study with *S. murinus* demonstrated that vagotomy did not reduce isoflurane-induced emesis, although isoflurane exposure produced a significant increase in c-Fos-positive cells in the hindbrain [19]. These results suggest that isoflurane triggers emesis via a direct action in the hindbrain in this animal. Indeed, 2-methylserotonin, a 5-HT3 agonist, was found to induce c-Fos expression in the AP of the least shrew [23]. Thus, the contribution of 5-HT3 receptors in emesis induced by volatile anesthetics remains controversial. Our present study showed that ondansetron partially reduced c-Fos expression, which does not contradict with the general clinical impression. Accordingly, it is important to consider the involvement of other mechanisms in volatile anesthetic-induced emesis, such as dopamine receptors, using this model in future studies.

This study also showed that isoflurane exposure did not affect kaolin intake in male rats. Yamamoto et al. [11] reported that sevoflurane induced pica in young female rats, but not in young male rats. Therefore, a gender difference might have contributed to these results. Alternatively, our results suggest that c-Fos expression in the AP may be a more sensitive response to emetic stimulation than pica in rats, regardless of the gender.

In conclusion, this is the first report to show that isoflurane induces c-Fos expression in the rat AP, which was partially inhibited by ondansetron pretreatment. Further study is needed to clarify the feasibility of this rat model to further explore the mechanism of emesis induced by volatile anesthetics.

References

1. Apfel CC, Kranke P, Katz MH, Goepfert C, Papenfuss T, Rauch S, Heineck R, Greim CA, Roewer N. Volatile anaesthetics may be the main cause of early but not delayed postoperative vomiting: a randomized controlled trial of factorial design. Br J Anaesth. 2002;88(5):659–68.
2. Stadler M, Bardiau F, Seidel L, Albert A, Boogaerts JG. Difference in risk factors for postoperative nausea and vomiting. Anesthesiology. 2003;98(1):46–52.
3. Apfel CC, Korttila K, Abdalla M, Kerger H, Turan A, Vedder I, Zernak C, Danner K, Jokela R, Pocock SJ, Trenkler S, Kredel M, Biedler A, Sessler DI, Roewer N. A factorial trial of six interventions for the prevention of postoperative nausea and vomiting. N Engl J Med. 2004;350(24):2441–51.
4. Diemunsch P, Joshi GP, Brichant JF. Neurokinin-1 receptor antagonists in the prevention of postoperative nausea and vomiting. Br J Anaesth. 2009;103(1):7–13.
5. King GL. Animal models in the study of vomiting. Can J Physiol Pharmacol. 1990;68(2):260–8.
6. Borison HL. Area postrema: chemoreceptor circumventricular organ of the medulla oblongata. Prog Neurobiol. 1989;32(5):351–90.
7. Miller AD, Leslie RA. The area postrema and vomiting. Front Neuroendocrinol. 1994;15(4):301–20.
8. Horn CC, Wallisch WJ, Homanics GE, Williams JP. Pathophysiological and neurochemical mechanisms of postoperative nausea and vomiting. Eur J Pharmacol. 2014;722:55–66.
9. Horn CC, Ciucci M, Chaudhury A. Brain Fos expression during 48 h after cisplatin treatment: neural pathways for acute and delayed visceral sickness. Auton Neurosci. 2007;132(1–2):44–51.
10. Batra VR, Schrott LM. Acute oxycodone induces the pro-emetic pica response in rats. J Pharmacol Exp Ther. 2011;339(3):738–45.
11. Yamamoto K, Yamamoto E, Sugimoto T, Sagakami T, Yamatodani A. Sevoflurane-induced pica in female rats. J Pharmacol Sci. 2016;131:68–71.
12. Yamamoto K, Asano K, Tasaka A, Ogura Y, Kim S, Ito Y, Yamatodani A. Involvement of substance P in the development of cisplatin-induced acute and delayed pica in rats. Br J Pharmacol. 2014;171:2888–999.
13. Takeda N, Hasegawa S, Morita M, Matsunaga T. Pica in rats is analogous to emesis: an animal model in emesis research. Pharmacol Biochem Behav. 1993;45:817–21.
14. Hunt SP, Pini A, Evan G. Induction of c-fos-like protein in spinal cord neurons following sensory stimulation. Nature. 1987;328(6131):632–4.
15. Miller AD, Ruggiero DA. Emetic reflex arc revealed by expression of the immediate-early gene c-fos in the cat. J Neurosci. 1994;14(2):871–88.
16. Reynolds DJ, Barber NA, Grahame-Smith DG, Leslie RA. Cisplatin-evoked induction of c-fos protein in the brainstem of the ferret: the effect of cervical vagotomy and the anti-emetic 5-HT3 receptor antagonist granisetron (BRL 43694). Brain Res. 1991;565(2):231–6.
17. De Jonghe BC, Horn CC. Chemotherapy agent cisplatin induces 48-h Fos expression in the brain of a vomiting species, the house musk shrew (*Suncus murinus*). Am J Physiol Regul Integr Comp Physiol. 2009;296(4):R902–R911911.
18. Parker RM, Bentley KR, Barnes NM. Allosteric modulation of 5-HT3 receptors: focus on alcohols and anaesthetic agents. Trends Pharmacol Sci. 1996;17(3):95–9.
19. Gupta RG, Schafer C, Ramaroson Y, Sciullo MG, Horn CC. Role of the abdominal vagus and hindbrain in inhalational anesthesia-induced vomiting. Auton Neurosci. 2017;202:114–21.
20. Horn CC. The medical implications of gastrointestinal vagal afferent pathways in nausea and vomiting. Curr Pharm Des. 2014;20(16):2703–3128.

21. Horn CC. Brain Fos expression induced by the chemotherapy agent cisplatin in the rat is partially dependent on an intact abdominal vagus. Auton Neurosci. 2009;148(1–2):76–82.
22. Mutoh T, Tsubone H, Nishimura R, Sasaki N. Effects of volatile anesthetics on vagal C-fiber activities and their reflexes in anesthetized dogs. Respir Physiol. 1998;112(3):253–64.
23. Ray AP, Chebolu S, Darmani NA. Receptor-selective agonists induce emesis and Fos expression in the brain and enteric nervous system of the least shrew (*Cryptotis parva*). Pharmacol Biochem Behav. 2009;94(1):211–8.