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Review

Role of the abdominal vagus and hindbrain in inhalational anesthesia-induced vomiting

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ABSTRACT

The incidence of postoperative nausea and vomiting (PONV) can be as high as 80% in patients with risk factors (e.g., females, history of motion sickness). PONV delays postoperative recovery and costs several hundred million dollars annually. Cell-based assays show that halogenated ethers (e.g., isoflurane) activate 5-HT_3 receptors, which are found on gastrointestinal vagal afferents and in the hindbrain - key pathways for producing nausea and vomiting. This project evaluated the role of the vagus and activation of the hindbrain in isoflurane-induced emesis in musk shrews, a small animal model with a vomiting reflex, which is lacking in rats and mice. Sham-operated and abdominal vagotomized shrews were exposed to 1 to 3% isoflurane to determine effects on emesis; vagotomy was confirmed by lack of vagal transport of the neuronal tracer Fluoro-Gold. In an additional study, shrews were exposed to isoflurane and hindbrain c-Fos was measured at 90 min after exposure using immunohistochemistry. There were no statistically significant effects of vagotomy on isoflurane-induced emesis compared to sham-operated controls. Isoflurane exposure produced a significant increase in c-Fos-positive cells in the nucleus of the solitary tract and vestibular nuclei but not in the area postrema or dorsal motor nucleus. These results indicate that the abdominal vagus plays no role in isoflurane-induced emesis and suggest that isoflurane activates emesis by action on the hindbrain, as shown by c-Fos labeling. Ultimately, knowledge of the mechanisms of inhalational anesthesia-induced PONV could lead to more targeted therapies to control PONV.

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1. Introduction

Postoperative nausea and vomiting (PONV) is a significant healthcare concern. The incidence of PONV, defined as nausea and vomiting experienced in the post-anesthesia care unit (PACU), can be as high as 80% in patients with risk factors, such as female sex, younger age, non-smoking status, and history of motion sickness (Apfel et al., 2012). Patients report PONV as one of the most distressing symptoms after surgery (Gan et al., 2003). The lack of effective ways to treat PONV delays patient discharge from the PACU and is estimated to cost the healthcare system several hundred million dollars annually (Gan et al., 2003; Habib et al., 2006). While current therapies have existed for decades, systematic reviews predict only 28% of those affected by PONV find benefit from these approaches (Carlisle and Stevenson, 2006).

PONV is primarily triggered by inhaled anesthetics and opioid analgesics (Apfel et al., 2012; Horn et al., 2014b). Inhaled anesthetics are known to cause PONV in the first two hours post-operatively (Apfel et al., 2002), coinciding with maximal symptomatology; however, little is known about the mechanism of inhalational anesthesia-induced PONV. Studies in cell-based assays indicate that halothane and isoflurane, inhaled anesthetics, enhance serotonin-sensitive 5-HT_3 receptor function (Machu and Harris, 1994; Parker et al., 1996) and halothane-induced emesis in musk shrews is substantially reduced by pre-treatment with the 5-HT_3 receptor antagonist ondansetron (Gardner and Perren, 1998). Although it must be verified with each specific halogenated ether, these observations suggest that these agents produce emesis via a 5-HT_3 mechanism. 5-HT_3 receptors are known to play an integral role in emesis and are found peripherally on abdominal vagal afferents and centrally in the area postrema (AP) and nucleus of the solitary tract (NTS) (Barnes et al., 1990; Kilpatrick et al., 1990; Marazziti et al., 2001; Reynolds et al., 1995).

The current studies were designed to test the effects of an ablation of the abdominal vagus on inhalational anesthesia-induced emesis and evaluate the activation of the hindbrain by isoflurane exposure. We used musk shrews (*Suncus murinus*) in these experiments because they have a vomiting reflex, which is lacking in mice and rats (Horn et al., 2013a); this species was also used in prior studies of inhalational anesthesia-induced emesis (Gardner and Perren, 1998; Horn et al., 2012). Here we used isoflurane as the emetic stimulus because this agent produces emesis in musk shrews (Horn et al., 2012) and in humans, similar in intensity to other halogenated ethers (e.g., sevoflurane, Apfel et al., 2002). c-Fos protein, a measure of neuronal activation (Sharp et al., 1993), was used in the current project to assess activation of the hindbrain areas believed to play a role in producing emesis, including the NTS and AP (Miller and Leslie, 1994; Yamada et al., 2000).

2. Materials and methods

2.1. Animals

In total, 48 experimentally naïve female musk shrews were used (>35 days of age, 20–50 g body weight; $n = 22$, Study 1, $n = 24$, Study 2, and $n = 2$, c-Fos anti-body testing); shrews were offspring from breeding stock obtained from the Chinese University of Hong Kong (a strain originating from Taiwan; Wang, 1994). Female animals were used because female sex is reported as a risk factor for PONV in clinical studies (e.g., Apfel et al., 2012). Animals were housed individually and fed a mixture of 75% Purina Cat Chow Complete Formula and 25% Complete Gro-Fur mink food pellets (Milk Specialty, New Holstein, WI; Temple, 2004). Musk shrews were maintained on a 12-h light/12-h dark cycle (lights on at 0700 h), with free access to food and water. Experiments were approved by the University of Pittsburgh's Institutional Animal Care and Use Committee, and animals were housed in an animal care facility accredited by the Association of Assessment and Accreditation of Laboratory Animal Care International.

2.2. Study 1: effect of abdominal vagotomy on isoflurane-induced emesis

2.2.1. Abdominal vagotomy surgery

Similar to a prior study (Horn et al., 2014a), shrews received a sham-operation or abdominal vagotomy by random assignment ($n = 11$ per group). Animals were anesthetized with isoflurane (2 to 3%) using an induction chamber followed by a nose cone during surgery. The ventral abdominal surface was shaved and the skin sterilized with betadine surgical scrub and 70% isopropyl alcohol. After a midline 1.5-cm laparotomy incision, the vagal trunks running along the esophagus were bluntly dissected and transected using a thermal cautery. The peritoneum was sutured (2–0 silk; Ethicon), and the skin was closed with surgical staples (7.5 × 1.75 mm, Michel). Sham operations were performed using a similar approach, except that the vagi were not manipulated or lesioned. Animals were weighed daily after surgery to assess body weight changes and were allowed to recover for 1 wk. before behavioral testing. For 2 days after surgery, animals received the analgesic ketoprofen (Sigma-Aldrich; 2 mg/kg sc, twice daily).

2.2.2. Behavioral testing

Four emetic tests (0, 1, 2, 3% isoflurane; Henry Schein) were conducted with 1 wk. between each test. Order of exposure was determined using a 4 × 4 Latin-square design (orders were 0 > 1 > 2 > 3%, 1 > 0 > 3 > 2%, 2 > 3 > 0 > 1%, and 3 > 2 > 1 > 0%). Isoflurane was provided from a calibrated isoflurane vaporizer (General Anesthetic Services Inc., South Park, PA, USA) with input of compressed 100% O_2 flowing at 6 l/min. 0% isoflurane tests consisted of 100% O_2. Gas was provided into a cylindrical induction chamber (10 × 8.5 cm, height and diameter of cylinder) and allowed to fill for 2 min prior to placing an animal inside (Horn et al., 2012). After 10 min of exposure in the induction chamber, shrews were transferred to the testing chamber (19 × 27 cm, width and length) for 30 min. Animal behavior was recorded using digital video cameras (Sony DCR-SR300 or HDR-XR550V, wide field lenses) positioned above the induction and testing chambers. While in the chambers, animals were observed and recorded for emetic episodes, abdominal contractions, body swaying, and sedation using keystrokes on a laptop computer running JWatcher software (http://www.jwatcher.ucla.edu; Blumstein and Daniel, 2007; Horn et al., 2013b). Emetic episodes in musk shrews occur as a series of closely spaced retches, which end with an expulsion phase (a retch = an abdominal

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