Physiology & Behavior 124 (2014) 129–137

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

Musk shrews selectively bred for motion sickness display increased anesthesia-induced vomiting

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HIGHLIGHTS

- Musk shrews were selectively bred (High & Low) for sensitivity to motion-induced emesis.
- Isoflurane exposure produced more emesis in High compared to Low strain animals.
- Results suggest a common mechanism for motion and inhalational anesthesia-induced emesis.

ARTICLE INFO

Article history:
Received 1 May 2013
Accepted 6 November 2013

Keywords:
Emesis
Vomiting
Nausea
Suncus
Genetics

ABSTRACT

Susceptibility to motion sickness is a predictor of postoperative nausea and vomiting, and studies in humans suggest that genetic factors determine sensitivity to motion sickness. The aim of the current study was to determine if a preclinical model could be selectively bred for motion-induced emesis and to assess a potential relationship to anesthesia-induced emesis. Musk shrews were tested for motion-induced emesis using a shaker plate (10 min, 1 Hz, and 4 cm of lateral displacement). Animals were rank ordered for motion-induced emesis and selectively bred to produce high and low response strains. Shrews were also tested with nicotine (5 mg/kg, sc), copper sulfate ($CuSO_4$; 120 mg/kg, ig), and isoflurane anesthesia (10 min; 3%) to measure responses to a panel of emetic stimuli. High response strain shrews demonstrated significantly more emetic episodes to motion exposure compared to low response strain animals in the F1 and F2 generations. In F2 animals, there were no significant differences in total emetic responses or emetic latency between strains after nicotine injection or $CuSO_4$ gavage. However, isoflurane exposure stimulated more emesis in F1 and F2 high versus low strain animals, which suggests a relationship between vestibular- and inhalational anesthesia-induced emesis. Overall, these results indicate genetic determinants of motion sickness in a preclinical model and a potential common mechanism for motion sickness and inhalational anesthesia-induced emesis. Future work may include genetic mapping of potential "emetic sensitivity genes" to develop novel therapies or diagnostics for patients with high risk of nausea and vomiting.

1. Introduction

As many as 35% of US adults have experienced vestibular dysfunction [1]. Motion sickness has significant adverse effects on cognitive and physical performance [2–5]. Although motion sickness involves several divergent brain pathways and functional components (e.g., pallor, cold sweating, and disorientation) and a link to pronounced activation of stress response systems [6–8], a cardinal feature is the activation of nausea and vomiting (NV) [9]. The incidence of NV in medical settings can reach 80%, particularly in individuals at highest risk, such as patients with a history of sensitivity to motion sickness [10,11], and a twin study estimates that motion sickness has a heritability of 53–70% [12]. Currently, anti-motion sickness drugs (e.g., histamine and muscarinic antagonists) do not always control NV and can result in sedation, blurred vision, and dizziness [13–15]. Research to date has focused on these older drugs, often with non-specific receptor targets, in heterogeneous human cohorts and preclinical models [14,15]. A high-throughput approach in an easily manipulated animal model could provide greater mechanistic insight and identify more effective therapeutic strategies for the control of motion sickness.

The focus of the current study was to selectively breed an animal model of motion-induced emesis that could be applied to future molecular-genetic studies. Musk shrews were used for these experiments

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http://dx.doi.org/10.1016/j.physbeh.2013.11.002

because they are capable of vomiting, unlike mice and rats [16,17], and are a well-characterized species for motion-induced emesis [18–24] using standardized behavioral test conditions [20]. Furthermore, musk shrews can be tested with this standardized approach in high-throughput screening (>40 animals per day); breed rapidly; have a short time to maturity (~35 days to adulthood); and, at 40 to 80 g, are only slightly larger than mice, which allows high density housing. Animals were tested for vestibular-induced emesis by placing test cages on a shaker plate (10 min, 1 Hz, and 4 cm of lateral displacement). Shrews were ranked from high to low emetic responses to motion and selectively bred to produce high and low response strains. Animals were also tested with nicotine (5 mg/kg, sc), copper sulfate ($CuSO_4$;120 mg/kg, ig), and isoflurane anesthesia (10 min; 3%) to determine responses to emetic stimuli acting on additional neural pathways. Circulating nicotine and intragastric $CuSO_4$ are believed to activate the area postrema and gastrointestinal vagal afferent pathways, respectively [25–29]; in contrast, little is known about the mechanism for anesthesia-induced emesis.

2. Materials and methods

2.1. Animals

Musk shrews were descendants from breeding stock obtained from the Chinese University of Hong Kong; a Taiwanese strain of *Suncus murinus* [30]. Studies used 30 females and 30 males in the parental generation, 16 females and 20 males in the F1 generation, and 15 females and 16 males in the F2 generation (a total of 127 animals). Animals were housed in clear plastic cages (28 × 17 × 12 cm), with a filtered air supply, under a 12 h standard light cycle (lights on: 0700 h), in a temperature (~23 °C) and humidity (~40%) controlled environment. Food and drinking water were freely available except during the brief test periods (~45 min). Food consisted of a mixture of 75% Purina Cat Chow Complete Formula and 25% Complete Gro-Fur mink food pellets [31]. All experiments were approved by the University of Pittsburgh Institutional Animal Care and Use Committee. Animals were housed in an Association for Assessment and Accreditation of Laboratory Animal Care International-accredited animal care facility.

2.2. Chemicals

Nicotine ((−)-nicotine, catalog # 36733) and $CuSO_4$ (copper (II) sulfate pentahydrate, catalog # 209198) were obtained from Sigma-Aldrich. Nicotine was made as a 2.5 mg/ml solution in sterile saline (0.15 M NaCl; subcutaneous injection = 5 mg/kg/2 ml), and $CuSO_4$ was dissolved in filtered water (Milli-Q) at a concentration of 24 mg/ml (gavage = 120 mg/kg/5 ml). Isoflurane was provided via an enclosed chamber (allowed to fill for 2 min before use). Flow rates of gas were 6 l/min (from a 100% O_2 compressed air canister flowing through an isoflurane vaporizer; Matrx, TEC-3). The percentage of isoflurane was set by the dial on the vaporizer and the enclosed induction chamber was 10 × 8.5 cm, height and diameter.

2.3. Emetic testing procedures

Emetic tests were conducted with a 3 to 4 week interval between tests to allow for recovery (test order: motion, nicotine, $CuSO_4$, and then isoflurane) [32,33]. Animals were tested between 0800 and 1200 h (light phase). Testing for males and females was balanced to control for time of day effects. For motion, nicotine, and $CuSO_4$ tests, animals received 15 min of adaption in the test chambers before the emetic stimulus. For motion exposure, the test chambers were 28 × 17 × 12 cm, with had a clear acrylic lid. These chambers were placed on a reciprocating shaker (Taitec, Double Shaker R-30, Taiyo Scientific Industrial). Horizontal motion (4 cm displacement; 2 cm left and 2 cm right; 1 Hz) was applied for 10 min based on prior studies of optimal parameters [20]. Animals were subcutaneously injected with nicotine (5 mg/kg) or gavaged with $CuSO_4$ (120 mg/kg), based on previous studies [21,34,35]. Cohorts of F1 and F2 generations were also tested for isoflurane-induced emesis. Only a subset of F1 animals were used for the isoflurane test because other members of this cohort were euthanized to collect blood for future genetic analysis. Animals were placed in a transparent induction chamber for 10 min of isoflurane exposure, and then transferred to a transparent observation chamber using our published procedure [32].

All animal behavior was recorded with a digital video camera (Sony DCR-SR300 or HDR-XR550V, wide field lenses) placed above each test chamber and connected to a computer for storage (Media Recorder; Noldus Information Technology). A trained observer was positioned outside the transparent test chambers to record the occurrence of an emetic episode (with or without a vomit), abdominal contraction, or a swaying movement using a notebook computer installed with coding software (JWatcher; http://www.jwatcher.ucla.edu/). Emetic episodes (with or without expulsion) were defined as a sequence of contractions of the abdominal region associated with forward movements of the head and separated by other episodes by a minimum of 2 s. In past studies, we have noted the occurrence of abdominal contractions (a single contraction of the abdominal region) and swaying movements (swaying the abdominal portion of the body from side to side) in association with emesis [36], and they were included in this report to determine potentially subtle differences between conditions.

2.4. Breeding procedures

Parental animals were ranked for emetic responses to motion exposure and divided into upper and lower 1/3 responders (n = 10/group for males and females). The cutoffs were ≥15 (High group) and ≤10 (Low group) episodes for males; and ≥13 (High group) and ≤7 (Low group) for females. These High and Low group motion response parental males and females (i.e., the P-split) were selectively bred (i.e., high with high, and low with low). Because of fewer animals in F1 breeding we used a High cutoff ≥13 emetic episodes for both males and females. To reduce the fixation of genes that are associated with inbreeding (and likely not with the phenotype of emesis), we only bred animals that were not related as siblings or parent and offspring. For breeding, one adult musk shrew female was placed in the home cage of an adult male overnight. The gestation period was approximately 30 days, with an average litter size of 2 pups. The pups were housed with their mother until 17 to 21 days of age; from that time, animals were housed singly and tested for emetic responses during adulthood (>35 days of age).

2.5. Data analysis

Dependent measures included total number of emetic episodes, episodes with vomiting, episodes without vomiting, duration (time from first to last emetic event), emetic rate (episodes/min), standard deviation of the emetic interval (SD-I), abdominal contractions, and swaying. SD-I was calculated as the standard deviation of the intervals between emetic episodes, and was used as a measure of the variability in the rhythm of emesis. In each generation, data were analyzed with ANOVA for each emetic stimulus and variable (strain by sex factorial design). Holm–Sidak tests were used to compare means after ANOVA. In the F1 isoflurane test, females and males were combined to form High and Low groups because of the lower power in this experiment (e.g., only n = 4 High females); *t*-tests were used to compare groups. It is frequently difficult to apply parametric statistics to behavioral latency data, which are often skewed. To address this issue we used survival plots and Cox regression analysis for comparison of latency data (time to the first emetic episode). This approach permits the use of all data, including censored values (i.e., animals that did not show emesis during the test period) [37]. $P < 0.05$ was used to determine statistical significance for all tests.