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Alpha-9 nicotinic acetylcholine receptors mediate hypothermic responses elicited by provocative motion in mice

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HIGHLIGHTS

- α9-AChR KO and WT mice were subjected to provocative motion and balance test.
- Motion stimuli caused hypothermic responses that were attenuated in KO mice.
- Motion stimuli caused cutaneous vasodilation that was attenuated in KO mice.
- KO mice required 25% more time to complete motor coordination/balance test.

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ABSTRACT

Hypothermic responses accompany motion sickness in humans and can be elicited by provocative motion in rats. We aimed to determine the potential role in these responses of the efferent cholinergic vestibular innervation. To this end, we used knockout (KO) mice lacking α9 cholinoreceptor subunit predominantly expressed in the vestibular hair cells and CBA strain as a wild-type (WT) control. In WT mice, circular horizontal motion (1 Hz, 4 cm radius, 20 min) caused rapid and dramatic falls in core body temperature and surface head temperature associated with a transient rise in the tail temperature; these responses were substantially attenuated in KO mice; changes were (WT vs. KO): for the core body temperature − 5.2 ± 0.3 vs. −2.9 ± 0.3 °C; for the head skin temperature − 3.3 ± 0.2 vs. −1.7 ± 0.2 °C; for the tail skin temperature + 3.9 ± 1.1 vs + 1.1 ± 1.2 °C. There was a close correlation in the time course of cooling the body and the surface of the head. KO mice also required 25% more time to complete a balance test. We conclude: i) that the integrity of cholinergic efferent vestibular system is essential for the full expression of motion-induced hypothermia in mice, and that the role of this system is likely facilitatory; ii) that the system is involvement in control of balance, but the involvement is not major; iii) that in mice, motion-induced body cooling is mediated via increased heat flow through vasodilated tail vasculature and (likely) via reduced thermogenesis. Our results support the idea that hypothermia is a biological correlate of a nausea-like state in animals.

1. Introduction

The phenomenon of motion sickness has been extensively studied and the currently accepted theory proposes that motion sickness develops when "sensory conflict" occurs between converging patterns of vestibular, visual, and proprioceptive inputs [10,11,18,32]. Precisely how these sensory modalities combine to produce motion sickness is not understood, but functional inner ear vestibular organs appear to be a prerequisite. For example, dogs are immune to motion sickness after bilateral labyrinthectomy [40], as are humans with bilateral vestibular deficiency [2,17]. It must be acknowledged that there is still some controversy in the field as sensitivity to both visual and vestibular stimulation has been reported in some individuals with bilateral vestibular deficiency [9,15]. A major component of vestibular organs is the sensory receptors or hair cells. These receptors convert (transduce) head movement into neural signals that are transmitted to the brain via the eighth cranial nerve. These signals play a crucial role in the pathogenesis of motion sickness and therefore modulation of hair cell output is likely to affect or even alleviate the deleterious effects of provocative motion.

Vestibular hair cells not only transmit signals to afferent nerves but also receive efferent input from the brainstem cholinergic 'group e', part of the *efferent vestibular system* or *EVS* [8,31,34]. Although the

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functional role of EVS in mammals is still poorly understood, preliminary evidence in mice [29] and more extensive non-mammalian studies suggest the EVS has a modulatory effect on hair cells [14]. This modulation of hair cells is achieved mainly through the principal EVS neurotransmitter, acetylcholine (ACh) [16] acting on nicotinic receptors containing the α9 subunit (α9-AChR). These particular receptors are found predominantly in the inner ear and specifically on the surface of type II hair cells [6,19]. In the current study we used wild type controls and the α9-AChR-knockout ($\alpha9^{-/-}$) mouse strain to explore the potential role of the EVS in physiological changes induced by provocative motion.

The major challenge in preclinical studies of motion sickness is objective assessment of its principal symptom, nausea. Most laboratory animal species do not possess vomiting reflex, and conclusions about a nausea-like state in such animals rely on behavioral indices with relatively low temporal resolution (e.g. pica [41]), or on those which require training paradigms (e.g. conditioned taste aversion [7], conditioned gaping or retching [28,39]), or utilize complex analysis of behavioral clusters [13]. Assessing emetic episodes in the relatively few common laboratory species with a vomiting reflex (dog, cat, ferret, house musk shrew) also has limitation as the pharmacology and neural substrates of nausea and emesis are different [36]. Our recent studies have demonstrated that provocative motion causes robust and prominent hypothermic responses in rats [4,12,24]. We contend that these falls in body temperature represent a biomarker of a nausea-like state in laboratory animals for four reasons: 1) these responses are provoked by motion and by chemical emetic stimuli [1,3,4,12,24]; 2) differential pharmacological sensitivity of these responses in rats mirrors sensitivity in humans [12]; 3) in house musk shrews, motion-induced hypothermia precedes emetic episodes [24]; and 4) there is a clear parallel in hypothermic responses between animals and humans in underlying physiological mechanism - cutaneous vasodilatation that favors heat loss [22,24].

It is currently unknown whether emetic stimuli trigger hypothermic responses in mice, and the first aim of our study was to test whether provocative motion reduces body temperature in this species, and to determine physiological mechanism that mediate this response. Second, comparing responses in α9-nAChR-knockout mice with wild-type controls, we addressed the question of whether these receptors influence afferent vestibular signals, and hence are potentially involved in the pathogenesis of motion sickness.

2. Methods

2.1. Experimental animals and ethics statement

As control animals, we used six background strain mice CBA/CaJ,129SvEvTac (CBA), the CBA strain was regularly refreshed using an SvEvTac mouse line to prevent genetic drift. We also used six homozygous α9-subunit knockout ($\alpha9^{-/-}$) mice (JAX005696; CBACaJ;129S-Chrna9tm1Bedv). The original $a9^{-/-}$ mice from Jackson Labs were back-crossed onto the CBA background control strain. Heterozygous offspring were used to produce a homozygous knockout line. The $\alpha9^{-/-}$ genotype was confirmed using standard PCR and the mutant primer sequence 'CAC GAG ACT AGT GAG ACG TG'. Body weights of the two mouse strains were 23 ± 1 (CBA) and 24 ± 1 g, ($\alpha9^{-/-}$). All procedures described below were approved by the University of Newcastle Animal Care and Ethics Committee, and complied with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

2.2. Surgery

Prior to all experiments, mice were housed in temperature-controlled room at 22 °C, under 12/12 h artificial light cycle. Water and food were available ad libitum. Animals were housed individually from the day of surgery to the end of the experiment. Surgical procedures were done under isoflurane anesthesia (1.5%) and body temperature was maintained using a heating pad. After a mid-abdominal incision, a telemetric transmitter (TA-F10, DSI, St Paul, MN, USA) was placed into the peritoneal cavity. Marbofloxacin and buprenorphine (0.05 mg/kg) were administered post-surgically. Animals were allowed to recover for one week.

2.3. Experimental procedures, data collection and analysis

On the day of experiment, the telemetric transmitter was magnetically activated, the mouse together with its home cage and a telemetric receiver, inserted under the cage, was placed on a laboratory orbital shaker (Model E0M6, Ratek, Australia). An infrared camera (FLIR-E50, Flir Systems, Wilsonville, OR, USA) was fixed above the cage. The cage top was removed, and cage height was extended to 50 cm by adding cardboard sides. This allowed an unobstructed view of the mouse from above, while preventing escape. A 15-min baseline activity recording (shaker OFF) was followed by 20 min of provocative motion (shaker ON, 1 Hz, 4-cm orbital displacement), and by 15-min recovery (shaker OFF). After the experiment, $\alpha9^{-/-}$ and CBA mice were either euthanized by an overdose of Xylazine or by cervical dislocation, to compare cooling rate observed during provocative motion with cooling rate following death.

The radiofrequency-modulated signal from the temperature transmitter was converted into an analog signal by means of the Temperature Analog Adaptor (DSI, St Paul, MN, USA) and acquired at 1 Hz using PowerLab-8s A/D converter and a Dell computer running Chart 7.0 software (ADInstruments, Sydney, Australia). Cranial skin and tail skin temperatures were measured off-line using ResearchIR software (Flir Systems, Wilsonvialle, OR, USA). Tail temperature was measured approximately 2 cm from the base of the tail; head skin temperature was measured from a circular region (8 mm diameter) at the middle of the head image. Infrared data were collected every 2 min; for compatibility, core body temperature data we averaged for 2-min periods.

2.4. Balance beam walk assay

Two separate groups of $\alpha9^{-/-}$ (n = 9) and CBA (n = 9) mice were trained to walk across a narrow beam (1800 mm in length, 12 mm wide, and mounted 600 mm from the floor as in [20]). On a separate day, mice of each genotype completed 4 trials of the balance beam walk task, with each trial being timed and the 4 trials averaged for each mouse.

2.5. Statistical analysis

We used the last time-point of baseline recording as reference point for statistical analysis. Statistical differences within groups were determined using repeated one-way ANOVA; statistical differences between groups - using repeated measures two-way ANOVA, with "time" and "group" factors, followed by *post-hoc* Bonferroni test. Pearson's correlation was used to assess temporal relations between the core body and head temperature values. For the balance beam walk assay, groups were compared by Students' *t*-test. All data are expressed as mean ± S.E.M. Differences are considered statistically significantly at $p < 0.05$. We used Prism v.6 (GraphPad, CA, USA) for all statistical analyses.

3. Results

Prior to the onset of motion stimuli, the temperature values for tail, core body, and head (T_{tail}, T_{core}, and T_{head}) did not differ between CBA and $\alpha9^{-/-}$ mice. Corresponding values were (CBA vs. $\alpha9^{-/-}$): 24.7 ± 0.2 vs. 24.5 ± 0.1 °C; 38.1 ± 0.2 vs. 37.8 ± 0.2 °C; and 32.5 ± 0.2 vs. 32.8 ± 0.2 °C for T_{tail}, T_{core}, and T_{head}, respectively; $p > 0.5$ for each pair. Within two minutes of provocative motion onset, infrared images of CBA mice showed that T_{tail} started to rise and reached a peak of 28.5 ± 1.0 °C within 6 min, and then gradually returned to the baseline