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Dexamethasone alleviates motion sickness in rats in part by enhancing the endocannabinoid system

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ABSTRACT

Low-dose dexamethasone has been widely used for the prevention of nausea and vomiting after chemotherapy and surgical procedures and to treat motion sickness due to its minimal adverse effects, but the mechanisms underlying its anti-motion sickness effects are poorly understood. Previous studies have demonstrated that the endocannabinoid system is suppressed by motion sickness but stimulated by dexamethasone. The aim of the present study was to determine whether dexamethasone has an anti-motion sickness effect in rats and to elucidate the mechanism of this action. We used HPLC–MS/MS to measure the plasma concentrations of anandamide and 2-arachidonoylglycerol + 1-arachidonoylglycerol, and we employed real-time quantitative PCR (qRT-PCR) and/or Western blot analysis to assay the expression of N-acylphosphatidyl-ethanolamine hydrolyzing phospholipase D, sn-1-selective diacylglycerol lipase, fatty acid hydrolase, monoacylglycerol lipase and endocannabinoid CB_1 receptor in the dorsal vagal complex and stomach of rats exposed to a motion sickness protocol. The results showed that dexamethasone lowered the motion sickness index and restored the levels of endogenous cannabinoids and the expression of the endocannabinoid CB_1 receptor, which declined after the induction of motion sickness, in the dorsal vagal complex and stomach.

1. Introduction

Motion sickness is a malady characterized by a combination of signs and symptoms, such as gasping, drowsiness, general inactivity and excessive vomiting, that accompany movement or perceived environmental movement (Oosterveld, 1995; Yates et al., 1998). Motion sickness can be triggered by many different stimuli, including traveling in automobiles, aircraft, spacecraft or boats and exposure to moving visual scenes (Money, 1970; Reason, 1978). A parabolic flight experiment showed that motion sickness was accompanied by a significant decrease in the reactivity of the peripheral endocannabinoid system (Chouker et al., 2010). A study of metabolic differences between subjects who experience nausea/vomiting upon acceleration and those who do not revealed that the level of arachidonic acid, the downstream metabolite of endocannabinoids, was significantly increased in nausea/vomiting subjects (Mo et al., 2012), suggesting that motion sickness may be associated with an impairment of endocannabinoid activity (Chouker et al., 2010).

Dexamethasone is often administered to cancer patients to counteract chemotherapy-induced vomiting, postoperative nausea/vomiting and analgesic requirements after thyroidectomy (Fujii and Nakayama, 2007), but its mechanism of action has not been clearly demonstrated (Malik et al., 2007; Hesketh, 2008). In 1986, Kohl proposed the use of dexamethasone to modulate motion sickness due to its long-acting, slow tolerance and fewer adverse effects compared with amphetamine and scopolamine (Kohl, 1986).

The endocannabinoid system comprises (i) cannabinoid receptors (cannabinoid type 1 receptor (CB1R), cannabinoid type 2 receptor and transient receptor potential vanilloid-1); (ii) their specific endogenous ligands (endocannabinoids), which include N-arachidonylethanolamine (anandamide, AEA), 2-arachidonoylglycerol (2-AG), noladin ether and virodhamine; and (iii) a number of biosynthetic and

Abbreviations: AEA, anandamide; ACTH, adrenocorticotropin; ACN, acetonitrile; 2-AG, 2-arachidonylglycerol; AP, area postrema; AVP, Arginine vasopressin; CIV, chemotherapy induced vomiting; CNS, central neural system; CRH, corticotropin releasing hormone; DAGL-a, sn-1-selective diacylglycerol lipase; DMNV, dorsal motor nucleus of the vagus; FAAH, fatty acid hydrolase; GAPDH, glyceraldehyde phosphate dehydrogenase; PONV, postoperative nausea and vomiting; PVN, paraventricular nucleus; TRPV1, transient receptor potential vanilloid-1; MAGL, monoacylglycerol lipase; NAPE-PLD, N-acylphosphatidyl-ethanolamine hydrolyzing phospholipase D; NTS, nucleus tractus solitarius; NR, nucleus of raphe; HPA, hypothalamic-pituitary-adrenal; Δ9-THC, delta-9-tetrahydrocannabinol

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degradation enzymes (De Petrocellis and Di Marzo, 2009). N-Acylphosphatidyl-ethanolamine hydrolyzing phospholipase D (NAPE-PLD) and sn-1-selective diacylglycerol lipase (DAGL-a) are responsible for the synthesis of AEA and 2-AG, respectively, whereas fatty acid hydrolase (FAAH) is responsible for the degradation of AEA, and monoacylglycerol lipase (MAGL) is responsible for the degradation of 2-AG (Pagano et al., 2008).

There are bidirectional and functional correlations between glucocorticoids and the endocannabinoid system. Glucocorticoids recruit the endocannabinoid system to exert rapid negative feedback control of the hypothalamic–pituitary–adrenal (HPA) axis during stress conditions (Evanson et al., 2010). AEA and 2-AG, as endogenous endocannabinoids at the terminals of vagal afferents in the gastrointestinal tract, have been demonstrated to participate in the complex regulation of food intake as well as emesis induced by toxins (Hu et al., 2007). Recently, researchers have found that AEA and 2-AG play important roles in the pathophysiology of nausea and vomiting induced by conditions such as migraine and cancer chemotherapy drugs and the conditioned gaping response elicited by a lithium-paired context (Rossi et al., 2008; Parker et al., 2009).

To understand the protective effect of dexamethasone against motion sickness in rats and determine whether this effect is mediated by stimulating the endocannabinoid system, we measured the levels of two major endocannabinoids – AEA and 2-AG – in plasma and their turnover enzymes in the dorsal vagal complex (DVC) and stomach. We analyzed the mRNA and protein levels of CB1R in these tissues to further elucidate the role of endocannabinoid signaling in the course of motion sickness.

2. Materials and methods

2.1. Animals and acceleration exposure

Thirty-three six-week-old male Sprague-Dawley (SD) rats weighing 190–240 g were purchased from Sino-British SIPPR/BK Lab Animal Ltd. (Shanghai, China). The animal protocols and procedures were approved by the Animal Use and Care Committee for Research and Education of the Second Military Medical University (Shanghai, China), and experiments were carried out in accordance with the guidelines published by the National Institutes of Health (USA) regarding the care and use of animals for experimental procedures. The animals were housed in Plexiglas box cages in the colony room at an ambient temperature of 22 ± 2 °C with a 12 h light/12 h dark schedule (lights on at 8 a.m.) and were maintained on an *ad libitum* schedule of food and water.

The rats were randomly assigned to four groups: the control group ($n=8$), which received an intra-gastric administration of water and, 30 min later, watched the acceleration apparatus and experienced the noise of the apparatus without experiencing actual acceleration; the acceleration model group ($n=8$), which received an intra-gastric administration of water 30 min before acceleration exposure; the DEX group ($n=9$), which received an intra-gastric administration of 0.05 mg/kg dexamethasone acetate 30 min before acceleration exposure, ensuring that the drug was completely absorbed according to the criteria defined by the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China); and the AM-251/DEX group ($n=8$), in which the animals were injected intraperitoneally with 5 mg/kg N-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (AM-251) (Tocris, Ballwin, MO) 15 min before the intra-gastric administration of 0.05 mg/kg dexamethasone acetate 30 min before acceleration exposure. Rats in the acceleration model, the DEX and AM-251/DEX groups were subjected to rotation. The dexamethasone acetate dosage and administration method were selected based on our previous experiments. During the 30 min rotation, the rats were given no food or tap water and were enclosed in a cuboidal Plexiglas box suspended on a metal frame, which revolved around an axis parallel to the floor as described by Cai et al. (2010). It has been demonstrated in our laboratory that this magnitude of stimulation can induce motion sickness in rats (Cai et al., 2010; Mo et al., 2012).

Dexamethasone acetate was dissolved in distilled water. AM-251 was dissolved in a vehicle composed of 2% DMSO plus 1% Tween-80 (Sigma-Aldrich, St. Louis, MO). Preliminary experiments showed that the motion sickness index induced by acceleration stimulation in gastric lavage-treated animals was not significantly different ($P > 0.05$) from the response of animals in the control group.

2.2. Observation of motion sickness symptoms

Because the rats had no emetic reaction, motion sickness symptoms were observed after rotation and recorded according to the motion sickness index, which reflects the severity of gastrointestinal symptoms caused by motion stimulation (Yu et al., 2007; Wei et al., 2011). The evaluation criteria of the motion sickness index were as follows: 1 point was given for each fecal granule, urination scored 1.2 points, severe piloerection scored 1.2 points, slight piloerection scored 0.6, and tremor scored 1.2 (Yu et al., 2007). For each criterion, 0 points were allotted if the symptom was not present. The motion sickness index was calculated by summing of all of these scores.

2.3. Tissue and blood preparation

Animals in each group were anesthetized by an intraperitoneal (*i.p.*) injection of 10% 0.3 g/kg chloral hydrate, and blood was drawn by cardiac puncture followed by a trans-cardiac perfusion of 60 ml chilled saline. A blood sample (5 ml) was collected by cardiac puncture using a BD-Falcon vacutainer. Blood samples were centrifuged at 3000 rpm for 20 min at 4 °C, and the plasma layer was collected and stored at −80 °C. The brain was immediately dissected and cooled in iced saline for 1 min before the dorsal vagal complex was carefully removed. The stomach was also removed for analysis. The dissected tissues were immediately frozen in liquid nitrogen and stored at −80 °C for further use.

2.4. HPLC–MS/MS

This analysis was performed on an LC/MS/MS system consisting of a Triple Quad™ 5500 (Applied Biosystems, Concord, ON, Canada), a SIL-HTc gradient pump (Shimadzu, Kyoto, Japan), two LC-20AD transfer pumps (Shimadzu, Kyoto, Japan), a DGU-20A3 vacuum pump (Shimadzu, Kyoto, Japan), a Gemini C18 chromatographic column (2.0 × 50 mm, 5 μm, Phenomenex, USA) and a C18 guard column (4.0 × 3.0 mm, 5 μm, Phenomenex, USA). Data acquisition and analysis were performed using Analyst software (version 1.5.1; Applied Biosystems/MDS Sciex). AEA, 2-AG, AEA d8 and 2-AG d8 were purchased from Cayman Chemicals (Nottingham, UK). Stock solutions were prepared at 0.1 mg/ml in acetonitrile for 2-AG, 2.5 μg/ml in acetonitrile for 2-AG d8, 0.5 mg/ml in methanol for AEA and 10 μg/ml in methanol for AEA d8. Stock and working solutions were stored at −80 °C or −20 °C. During sample preparation, all steps were carried out at 4 °C to prevent the isomerization or degradation of the compounds. After spiking with the internal standard, 300 μl of the plasma sample/calibration sample was treated by the liquid–liquid extraction method with 1 ml hexane/ethyl acetate (1:1, v/v) by vortex shaking (approximately 1500 moves/min) for 10 min followed by centrifugation at 15,000 rpm