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The saturated fatty acid, palmitic acid, induces anxiety-like behavior in mice☆

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ABSTRACT

Objectives. Excess fat in the diet can impact neuropsychiatric functions by negatively affecting cognition, mood and anxiety. We sought to show that the free fatty acid (FFA), palmitic acid, can cause adverse biobehaviors in mice that last beyond an acute elevation in plasma FFAs.

Methods. Mice were administered palmitic acid or vehicle as a single intraperitoneal (IP) injection. Biobehaviors were profiled 2 and 24 h after palmitic acid treatment. Quantification of dopamine (DA), norepinephrine (NE), serotonin (5-HT) and their major metabolites was performed in cortex, hippocampus and amygdala. FFA concentration was determined in plasma. Relative fold change in mRNA expression of unfolded protein response (UPR)-associated genes was determined in brain regions.

Results. In a dose-dependent fashion, palmitic acid rapidly reduced mouse locomotor activity by a mechanism that did not rely on TLR4, MyD88, IL-1, IL-6 or TNFα but was dependent on fatty acid chain length. Twenty-four hours after palmitic acid administration mice exhibited anxiety-like behavior without impairment in locomotion, food intake, depressive-like behavior or spatial memory. Additionally, the serotonin metabolite 5-HIAA was increased by 33% in the amygdala 24 h after palmitic acid treatment.

Conclusions. Palmitic acid induces anxiety-like behavior in mice while increasing amygdala-based serotonin metabolism. These effects occur at a time point when plasma FFA levels are no longer elevated.

1. Introduction

Overweight/obesity is associated with a variety of organic comorbidities including cardiovascular disease, stroke, type 2 diabetes (T2D) and cancer [1,2]. Recently, neuropsychiatric complications such as depression, cognitive impairment and anxiety are seen as serious adverse sequelae in overweight/obese individuals [3–5]. Over-nutrition due to an excess intake

Abbreviations: HFD, high-fat diet; T2D, type 2 diabetes; FFA, free fatty acids; TLR, Toll-like receptor; IL, interleukin; MyD88, myeloid differentiation primary response gene (88); DA, dopamine; NE, norepinephrine; NME, normetanephrine; 5-HT, serotonin; 5-HIAA, 5-hydroxyindoleacetic acid; HVA, homovanillic acid; DOPAC, 3,4-dihydroxyphenylacetic acid; KO, knockout; UPR, unfolded protein response.

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of dietary fat is causally linked to the overweight/obese state [6,7], and more recently to metaflammation-linked neurodegenerative disease [8] and adult obstructive airway disease-associated, cognitive impairment, depression and anxiety [5,9,10]. More recently, high-fat diet (HFD)-induced over-nutrition is tied to neuropsychiatric morbidities prior to the onset of inflammation, hyperglycemia and weight gain [11,12] indicating that excess fat in the diet is, itself, harmful to psychological health.

One consequence of an HFD is an increase in circulating free fatty acids (FFAs) [13]. Canonically, elevated plasma FFAs are associated with insulin resistance, non-alcoholic fatty liver disease (NAFLD), pre-diabetic neuropathy, decreased aortic distensibility, and ischemic stroke [14–19]. Much less, however, is known about the impact of FFAs on the brain and behavior outside of an impact on food intake [20]. We recently demonstrated that a short-term HFD in mice impairs object-based memory and causes anxiety-like behavior after 1 week of feeding, suggesting that an HFD can negatively impact amygdala-related processes in a relatively rapid fashion [12]. Since the brain is rich in receptors that can recognize FFAs including Toll-like receptors (TLRs) [21] and free fatty acid receptors (FFARs) [22,23], FFAs have a real potential to modulate higher brain function.

Anxiety disorders, including generalized anxiety disorder (GAD), obsessive-compulsive disorder (OCD), panic disorder and post-traumatic stress disorder (PTSD) are among the most commonly reported neuropsychiatric conditions with a lifetime prevalence of nearly 29% [24]. Organically, how anxiety develops is poorly understood but metabolic interventions (exercise [25], weight loss [26]) and diet modification (removal of caffeine [27] and alcohol [28]) are often suggested as ways to reduce and/or stave off GAD or panic disorder. Given that palmitic acid is the most abundant saturated fatty acid in most diets [29] and its elevation in plasma is associated with poor clinical outcomes and disease progression for both the metabolic syndrome and obesity [30,31], the question addressed here is whether FFAs can negatively impact biobehaviors in mice.

2. Methods

2.1. Materials

All reagents and chemicals were purchased from Sigma-Aldrich. All primers were purchased from Applied Biosystems.

2.2. Animals

Animal use was conducted in accordance with Institutional Animal Care and Use Committee approved protocols at the University of Illinois. Wild-type C57BL/6J (WT), TLR4 knockout (KO), myeloid differentiation primary response gene 88 (MyD88) KO, interleukin (IL)-1 receptor-1 (IL-1R1) KO, IL-6 (IL-6) KO, and tumor necrosis factor-α (TNFα) KO mice (all on a C57BL background) were originally purchased from The Jackson Laboratory (Bar Harbor, ME). Mice were group housed (8 per cage) in standard shoebox cages and provided water and food ad libitum, then moved to individual housing in standard shoebox cages the day prior to treatment unless noted otherwise. Housing temperature (72 °F) and humidity (45–55%) were controlled as was a 12/12 h reversed dark–light cycle (2100–0900 h). Video recording of animal behavior was performed under red light using a Sony HDR-XR500V Night Shot capable video camera (Tokyo, Japan). All treatments at each time point represent separate cohorts of mice, except locomotor activity which was a repeated measure. Mice used were between 8 and 16 weeks of age. All behavior testing was completed in the dark cycle under red light illumination unless otherwise indicated. A total of 382 mice were used.

2.3. Injectables

FFAs (palmitic acid, palmitoleic acid, myristic acid, decanoic acid, octanoic acid, valeric acid) were administered IP in a vehicle of castor oil at a volume of 50 μl/mouse. Palmitic acid was administered for the dose response at 0.3, 3, and 30 μmol/mouse, after which all free fatty acids (FFAs) were tested at 30 μmol/mouse. All FFA injections occurred at the onset of the dark cycle.

2.4. Spontaneous locomotion

Locomotion was measured by videography in conjunction with automated video tracking software (Noldus Information Technology EthoVision XT 7 (Leesburg, VA)), as we have described [32,33]. At times indicated after treatment, mice were video recorded in their home cage for 5 min. Distance moved (cm) was determined, and results are presented as percent vehicle control.

2.5. Object investigation

As we have described [33], the subject mouse was placed in a novel home cage-sized arena with two objects 10 cm apart at one end. Mice were video recorded for 10 min and investigative behavior of the objects was evaluated using EthoVision XT 7. Results were expressed as raw investigation time (s).

2.6. Zero maze

As previously described [12], mice were transferred from their home cage to the high wall section of a Zero maze, which was divided into 4 quadrants, 2 of which had high walls (14 cm). Testing was completed under white light illumination and video recorded for 5 min. Time spent in the non-high wall area (open arm) was quantified as we have described [33].

2.7. Food intake

As we have described [33], prior to treatments and, at each time point indicated, food was weighed. Food intake was calculated as the difference in weight of food removed from the feed bowl.

2.8. Forced swim test

The test mouse was transferred from its home cage to a clean white cylindrical PVC container (diameter 16 cm; height 31 cm)