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Research report

High fat diet induced-obesity facilitates anxiety-like behaviors due to GABAergic impairment within the dorsomedial hypothalamus in rats

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HIGHLIGHTS

- HFD facilitates the development of anxiety-related behavior.
- Muscimol intra-dorsomedial hypothalamus has an anxiogenic effect in obese rats.
- BMI intra-dorsomedial hypothalamus does not cause an anxiogenic effect in obese rats.
- Memory acquisition and locomotor activity are not altered by GABAergic manipulation.

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ABSTRACT

Overweight and obesity are conditions associated with an overall range of clinical health consequences, and they could be involved with the development of neuropsychiatric diseases, such as generalized anxiety disorder (GAD) and panic disorder (PD). A crucial brain nuclei involved on the physiological functions and behavioral responses, especially fear, anxiety and panic, is the dorsomedial hypothalamus (DMH). However, the mechanisms underlying the process whereby the DMH is involved in behavioral changes in obese rats still remains unclear. The current study further investigates the relation between obesity and generalized anxiety, by investigating the $GABA_A$ sensitivity to pharmacological manipulation within the DMH in obese rats during anxiety conditions. Male *Wistar* rats were divided in two experimental groups: the first was fed a control diet (CD; 11% w/w) and second was fed a high fat diet (HFD; 45% w/w). Animals were randomly treated with muscimol, a $GABA_A$ agonist and *bicuculline methiodide* (BMI), a $GABA_A$ antagonist. Inhibitory avoidance and escape behaviors were investigated using the Elevated T-Maze (ETM) apparatus. Our results revealed that the obesity facilitated inhibitory avoidance acquisition, suggesting a positive relation between obesity and the development of an anxiety-like state. The injection of muscimol (an anxiolytic drug), within the DMH, increased the inhibitory avoidance latency in obese animals (featuring an anxiogenic state). Besides, muscimol prolonged the escape latency and controlling the possible panic-like behavior in these animals. Injection of BMI into the DMH was ineffective to produce an anxiety-like effect in obese animals opposing the results observed in lean animals. These findings support the hypotheses that obese animals are susceptible to develop anxiety-like behaviors, probably through changes in the GABAergic neurotransmission within the DMH.

1. Introduction

Overweight and obesity have become a critical worldwide health problem on the last decades [1–3]. These conditions are associated with several adverse clinical health consequences, such as type 2 diabetes [4], cardiovascular diseases [5] and others comorbidities with an overall mortality increase [3,5]. Moreover, obesity has been linked to neuropsychiatric and anxiety disorders, including generalized anxiety disorder (GAD), panic disorder (PD), post-traumatic stress disorder (PTSD), emotional reactivity and cognitive dysfunctions [6–8].

Studies have shown a positive relation among obesity, psychiatric disturbance and physiological arousal. In fact, our group have recently shown that high fat diet (HFD; 45% lard fat) induced obesity rats, showed an exacerbated cardiovascular response during emotional stress [9,10]. Studies showed that, HFD consumption by

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the mother during the perinatal period also promoted anxiety like behaviors [11,12]. Moreover, premature exchange of breastfeeding to a HFD in the postnatal period induced the development of anxiety-like behaviors in the offspring tested in the Elevated Plus Maze (EPM) [12]. However, the influence of adulthood obesity as a relevant cause for the dysregulation of brain circuits, which could lead to anxiety-like disorders, is not completely understood.

Converging lines of evidence suggest that the dorsomedial hypothalamus (DMH) is a crucial brain region involved in the regulation of behavioral responses, particularly fear, anxiety and panic-like disorders [13–16]. The medial hypothalamus is not only involved in behavior regulation, but also in physiological functions such as reproduction, food ingestion, metabolism and environmental threats [17,18]. In this context, the DMH has been shown to act as an important neuroanatomical substrate in coordinating behavioral changes [9,19]. Previous studies have shown that the DMH neuroactivity is essential in regulating anxiety conditions [13,14,20]. It is important to highlight that the activity level within this region depends on a balance of excitatory (Glutamate) and inhibitory (GABA) neurotransmitters [21,22]. In fact, injection of the $GABA_A$ agonist muscimol into the DMH evokes a panicolytic effect, which was evidenced by the increased escape latency on the Elevated T-maze (ETM), but was ineffective to change the learned conditioned anxiety-like response [17]. Notwithstanding, direct microinjections of $GABA_A$ antagonist *bicuculline methiodide* (BMI) into the DMH have an anxiogenic effect in animals tested in the Elevated Plus Maze (EPM) [13,20,23].

In a previous work we tested the relation between the cardiovascular reactivity during emotional stress and GABAergic tonus within the DMH in obesity-induced rats. As reported in the literature, we observed that a muscimol injection into the DMH was effective to control mean arterial pressure (MAP) and heart rate (HR) increases caused by stress in control animals. Nevertheless our results have shown that muscimol was ineffective to reduce the increase in MAP and HR induced by air jet stress in rats fed a high fat diet. Moreover, the injection of BMI into the DMH of obese rats evoked only a transient increase in MAP and HR when compared to control animals. We believe that the ineffectiveness of these drugs (muscimol and BMI) in obese rats might be due to a blunted-GABAergic tonus within the DMH, which could be the main cause for the exacerbated response observed in these animals during emotional stress test (see Ref. [10]).

Mindful that obese animals seem to have a blunted inhibitory GABAergic tonus within the DMH, and that functional changes in the DMH are related to mood disorders, we hypothesized that obese rats could be susceptible to develop anxiety and/or panic-like disorders due to changes in GABA activity within this nucleus.

Thus, the current study further investigates the relation between obesity and generalized anxiety, by investigating the importance of the impairment of $GABA_A$ inhibition within the DMH observed in obese rats on anxiety conditions. For that, we used the elevated T-maze model (ETM), an apparatus capable to investigate two different behavioral responses. The ETM investigates the conditioned fear, associated with GAD, through inhibitory avoidance behavior. When the animal is placed in the enclosed arm of the ETM, they cannot see beyond the walls, unless it pokes its head outside the arm. Further, the ETM is capable to investigate an unconditioned fear, which is associated with PD, through measuring escape latency. Rats seem to have an innate fear of open and high spaces. Accordingly, we used this apparatus to assess anxiety-like and panic-like responses in obese and lean animals, during controlled conditions, and after the administration of the $GABA_A$ receptor agonist muscimol or $GABA_A$ receptor antagonist BMI into the DMH.

2. Methods

2.1. Animals

Male Wistar rats (CEUA #2013/33, Federal University of Ouro Preto) weighing 100 ± 10 g were housed in groups of four in a polypropylene cage during 9 weeks, under controlled temperature (23 ± 1 °C), and in a 12:12 h dark/light cycle with ad libitum water and food. After the surgical intervention, animals were individually housed until full recovery for the behavioral testing. The Ethical Committee for Animal Research at Ouro Preto Federal University has approved all the procedures in this study, according to Brazilian Society of Neuroscience and Behavior and the "National Institutes of Health Guidelines for the Care and Use of Laboratory Animals" (8th edition; 2011).

2.2. Diet

Animals were divided into two experimental groups, one received either a control diet (CD) composed by 11% w/w fat (Nuvilab, Brazil) and the other received a high fat diet (HFD), composed by 45% w/w fat [10] (based on a D12451 formula sold by Research Diets, Inc., New Brunscwick, NJ, USA) [24,25]. Changes in body composition caused by HFD feeding during the diet protocol period of 9 weeks were defined according to Adiposity and Lee Indexes (AI; LI). To calculate the LI, the weight cubic root (g) was divided by nose-anal length (cm). For AI, the sum of visceral fat weight (took from the epididymal, retroperitoneal and inguinal tissues) was divided by final rat weight and the result was multiplied by 100.

2.3. Apparatus

To evaluate behavioral changes, animals were tested in the Elevated T-Maze (ETM). An apparatus made of wood and formed by three arms of equal dimensions (50 cm × 12 cm) 50 cm far from the floor. The perpendicular arm of the ETM was enclosed by a 40 cm high wall, and the two opposite open arms were surrounded by a 1 cm pexiglass rim (aimed to avoid animals' falls). To evaluate the locomotor activity, animals were tested in an Open Field square arena (OF; 40 cm l × 32 cm h) made of polypropylene equally divided in 16 squares on the floor. Room luminosity was set at 60 Lux frequency on the ETM and OF center. Following each behavioral tests routine, the equipment was cleaned with 20% ethanol.

2.4. Surgery procedures

After 9 weeks of diet, animals were submitted to a surgical procedure to implant stainless steel guide cannulas, unilaterally or bilaterally, into the DMH to enable drugs microinjection [26]. Briefly, rats under anesthesia (80 mg kg^{-1} Ketamine; 11.5 mg kg^{-1} xylazine, i.p, supplemented if needed) were positioned in a stereotaxic frame, with the incisor bar positioned 3.3 mm below the interaural line. A small craniotomy was made near the bregma reference point to allow cannula insert (15 mm length; stereotaxic DMH coordinates: AP −3.2; LL ±0.6; DV −7.6 mm) according to Paxinos and Watson atlas [27]. The cannula was fixed in the skull with the aid of two stainless steel screws and acrylic resin. Following surgery rats received analgesics (ketoflex 4 mg/kg, 0.1 mL/300 g s.c., Mundo Animal, Brazil) and preventive treatment against infection (penthabiotic, 0.2 mL/100 g s.c., Fort Dodge Animal Health, Brazil). Animals were re-allocated to individual cages and left quiescent for 6 days after surgery, until the experimental procedures, except for normal cage cleaning.