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ORIGINAL ARTICLE

Methyl Jasmonate Modulates Feeding Behaviors and Hypothalamic Expression of the Orexin 1 Receptor in Rats

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ABSTRACT

Objectives: Active plant ingredients have been successfully used in modern medicine to control appetite and energy hemostasis. This study was designed to evaluate the efficacy of the phytohormone methyl jasmonate (MJ) on food-related behaviors in rats.

Materials and Methods: Adult male Wistar rats were randomly divided into different groups (7 rats) and infused intracerebroventricularly (*i.c.v.*) with MJ vehicle (DMSO) or MJ (2.5, 5 and 10 µg/rat). Then, the individual rats were placed in an automated open field-like apparatus to assess a 12-h food-related activity in light and dark times. After behavioral tests, immunofluorescence staining of the orexin 1 receptor (Orx1R) was studied in the hypothalamus of rats.

Results: MJ (2.5, 5, and 10 µg/rat) administration significantly decreased food intake in the light and dark phases compared with the control group. Moreover, all the MJ-treated groups exhibited a decrease in visits to food containers at the light and dark times ($p < 0.001$). In addition, rats infused with MJ at 5 µg and 10 µg spent less time in the ports of food containers in the light and dark phases in comparison with control rats. Time in zone-related to food and locomotor activity was significantly decreased in the MJ (5 µg) groups during the light time and in all MJ-injected groups in the dark time. Moreover, hypothalamic expression of Orx1R in rats treated with MJ (5 µg) was significantly lower as compared to the control group.

Conclusion: Overall, the results indicated the potential of MJ to modulate feeding-related behavior and Orx1R expression in the hypothalamus of rats.

Key words: Methyl jasmonate, feeding behavior, Ox1R, rats

INTRODUCTION

Feeding behavior and energy consumption have been considered important health concerns in humans. In particular, plant-based diets have gained scientists' attention in recent decades. Plant ingredients have been widely used in the food industry to control appetite, body weight, metabolic disturbances, and energy intake.[1,2]

The plant hormone methyl jasmonate (MJ) was initially isolated from the floral scent of jasmine plant. The growing body of evidence shows its value in modulating neurologic processes in animals.[3-6] It is structurally similar to anti-inflammatory prostaglandins (PGE2), which could decrease the inductin of interleukin-6 (IL-6), nitric oxide, and tumor necrosis factor (TNF-α).[7] It has been shown that MJ attenuates depression-like behavior, anxiolytic responses, and learning and memory decline in rodents.[3,5,8] Moreover, MJ was able to decrease stress oxidative indices in the brains of mice.[9,10]

In addition, jasmonate is usually ingested with plant nutrients and may elicit physiological competence or toxic effects when ingested at high dosages.[11] IMJ is present in plant foods in

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different concentrations, so its intake is strongly influenced by cultural and social nutritional behaviors.[12]

In different areas of the brain, neuromodulators are also intricately involved in energy expungers and food intake.[13,14] Studies have emphasized the key role of hypothalamic orexin peptides, *i.e.* orexin-A and orexin B, in appetite and metabolic procedures. These neuropeptides act by activating two G-protein-coupled receptors, including the orrin 1 receptor (Orx1R) and orrin 2 receptor (Orx2R). Orexin-A is equal at both receptors; however, orexin-B exhibits more competence to Orx2R. It has been shown that diet intervention is related to changes in OrxR expression in the rat brain.[15,16]

Although MJ is found in most dietary plant sources,[17] there is a lack of study to report the capability of MJ to modulate feeding behavior. This study was designed to evaluate, whether central administration of MJ can modulate feeding-associated behavior in rats or not. Moreover, alteration in OrxRs expression in the hypothalamus was evaluated in MJ-infused rats.

MATERIALS AND METHODS

Animal

Adult male Wistar rats weighing 230-270 g were divided into different groups. The rats were caged under controlled light/dark cycle (12/12 h) conditions and constant temperature (22 ± 2 ˚C). The diet and water were available at all times. All rats were habituated to lab environment for 30 min *per* day in the cage for a week and then valued.

Surgery

Ketamine (60 mg/kg) and xylazine (5 mg/kg) anesthetized rats were placed in a stereotaxic device (Stoelting, USA). A 23-gauge stainless steel guide cannula was bilaterally inserted into the lateral ventricles. The stereotaxic coordinates were derived from Paxinos and Franklin[18] atlas (AP = 1.6 mm, ML = ± 0.8, and DV = 3.4 mm). The cannulas were then attached to the skull using two screws and dental acrylic. Rats were recovered for 7 days in separate cages before the initiation of experiments.

Drug

MJ (purity > 95%) was bought from Sigma-Aldrich and sodium chloride 0.9% *w/v* was diluted.

Microinjection

Microinjections were accomplished with a Hamilton syringe (1 µL) connected to a needle (27-gauge) *via* a polyethylene tube. Drug infusion was performed at a rate of 1 µL/min/rat/ side.

Immunofluorescence

Paraffin blocks through the hypothalamic nuclei were sectioned and deparaffinized. The sections were treated for 30 min for antigen retrieval by hydrochloric acid solution (2%). After 5 min at room temperature, the samples were neutralized by incubation in 0.1 M sodium borate buffer, and after 30 min, they were washed in phosphate buffer saline (PBS). The primary antibody diluted (1 in 100) with PBS was added to the samples, and they were then placed in a refrigerator at 2 to 8 ˚C for 24 h to creating a humid environment to prevent tissue drying. After 24 h, the brain tissue was removed from the refrigerator and washed 4 times with PBS for 5 min each time. The secondary antibody was then added at a dilution of 1 to 150 and incubated in a 37 ˚C incubator for 90 min in the dark. The sample was transferred after 3 washes from the incubator to a dark room, we added DAPI (Sigma-D9542). Twenty minutes later, the samples were washed with PBS. Glycerol and PBS solution were poured on the sample and Orx1R immunoreactivity in each section was observed using a fluorescence microscope. Using a digital camera the microscopic images (x40) enclosing the population of Orx1R immunoreactive neurons in each section were taken.

Rat preference meter device

A square automated device (60 x 60 cm) with 30 cm high black plexiglass was used. The floor was separated into nine equal squares. For recording and monitoring the rats' location and food and water consumption, the apparatus was equipped with underneath load sensors. The animal was released from the central square (square 5) for assaying preference behavior. The four middle squares show the preference for the content of the nearest container. Also, the corner squares have been considered for resting animals. Detailed visual cues that help the rat remember taste memory were also assimilated (Figure 1).

Experimental design

Seventy food-deprived (12 h) rats were randomly divided into ten groups (n: 7) as follows: untreated control, sham-operated that was cannulated and infused with MJ vehicle (DMSO), and MJ-treated groups that were cannulated and injected with three different doses of MJ (2.5, 5, and 10 µg/rat) in two phases of light and dark. Food-related activities were evaluated using an automated system. Total food consumption, number of visits, time spent, and distance traveled to food ports and zones were calculated using software. In the habituation trial, the animals were allowed to freely explore the device two days (15 min/day) before the test. The rats were released when one container was provided by a 30 g normal pellet and allowed food intake within a 12-h period (separately in two phases day and night). In the habituation test, the animal was discarded from the experiment, when it spent more time in a particular zone or did not show probing behavior.[19]

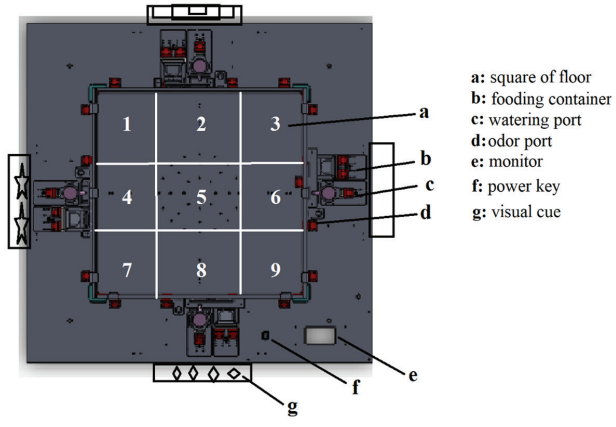

Figure 1. Overlook of the rat preference meter apparatus

Statistical analysis

All behavioral data were calculated using SPSS software. The data are expressed as means ± standard error of the mean. A two-way ANOVA was applied for analyzing the data. $P < 0.05$ was considered significant.

RESULTS

Food consumption

Figure 2 describes the amount of food intake in different groups of rats. Rats treated with MJ showed a significant decrease in food consumption in both light and dark phases compared with control and sham groups. The lowest amount of food consumption in the light and dark phases was indicated in the MJ-injected groups at 5 and 10 µg, respectively ($p < 0.001$).

Number of visits

There were significant differences between the control and MJ (2.5, 5, and 10 µg) groups in the number of total entries to the food ports and zones. The MJ groups showed a decrease in the number of visits in the light and dark phases ($p < 0.001$) as compared to the control group (Figure 3). In all groups, entries to food ports and zones were significantly increased in the dark phase compared to the light phase (Figure 3).

Time spent in the port and zone

Figure 4A presents that the time spent in the food port in the MJ-treated groups (5 and 10 µg), was significantly lower than that in the control group. The overall amount of time spent in the food zone was significantly decreased in MJ-infused rats at 5 and 10 µg in the light and dark phases (Figure 4B).

Locomotor activity

The distance traveled in the light phase in the MJ-treated group (5 µg) was significantly lower than that in the control group ($p < 0.05$). In the dark phase, there were increases in traveled distance in the groups of rats treated with MJ at 2.5, 5, and 10 µg as compared to control and sham rats. In addition, the distance traveled in the dark phase was significantly increased compared with that in the light phase ($p < 0.001$) (Figure 5).

IHC

Immunofluorescence staining of Orx1R in hypothalamic nuclei, including the ventral arterial thalamic nucleus (VA) and anterior hypothalamic arc (AHC), was achieved using antibodies directed against Orx1R combined with DAPI nucleus staining. Figure 6, panels A and B, display representative sections taken from the atlas of Paxinos and Watson, and immunofluorescence of Orx1R-positive cells in the hypothalamic nuclei, respectively, in control and MJ (5 µg) treated groups. In Figure 6, panel C, the numbers of Orx1R-positive cells in the hypothalamic nuclei VA (graph A) and AHC (graph B) were significantly attenuated in MJ-treated animals (5 µg) as compared to the control group ($p < 0.01$).

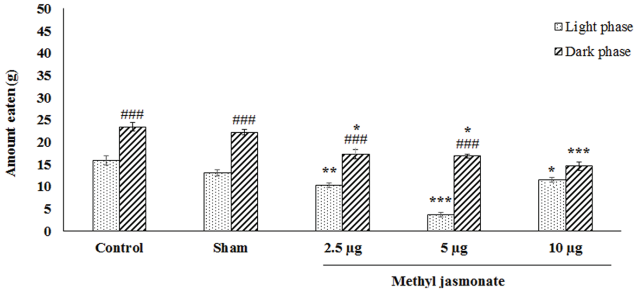

Figure 2. Effect of MJ (2.5, 5, and 10 µg/rat) on food consumption (in grams) in light and dark time (n: 7). Data are presented as mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ *vs* control and sham groups in light phase ###$p < 0.001$ *vs* control and sham groups in the dark phase

DISCUSSION

The data of this study indicated that central administration of MJ can attenuate feeding-related behaviors in adult male rats. The behavioral effects were accompanied by Orx1R downregulation in the hypothalamus of rats.

Here, an automated open-field box was used to monitor the feeding behavior of rats in a 12 h light and 12 h darkness cycle.[20] The acknowledged characteristics included the amount of food consumed, time spent, number of visits, and distance each rat traveled in ports and zones of food containers.[19] In line with previous studies on nocturnal animals, the highest nurturing activities were found in the darkness time.[21]

This study was the first to report MJ intervention on feeding behavior in rats. However, previous studies have emphasized the efficiency of MJ in modulating some neuronal processes, including learning and memory, anxiety-like behavior, stress, and nociception.[8,22,23]

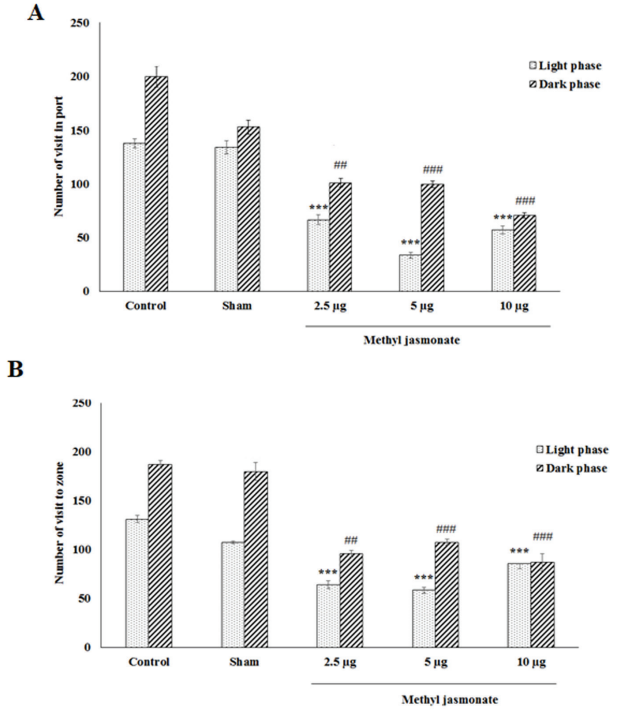

Figure 3. Effect of MJ (2.5, 5, and 10 µg/rat) on the number of visits to different ports (a) and zones (b) during light and dark time (n: 7). Data are presented as mean ± SEM. *$p < 0.05$, ***$p < 0.001$ vs control and the same groups, ##$p < 0.01$, ###$p < 0.001$

There are few data available showing MJ involvement in feeding behaviors. In rats suffering from arthritis and healthy rats, oral administration of MJ for 18 consecutive days increased the activity of mitochondrial $NADP^+$-dependent enzymes and decreased the levels of glucose flux through glycolysis in the liver. Regarding MJ effects to decrease hepatic glucokinase activity and glycolysis, it potentially might increase mitochondrial ROS production.[24,25]

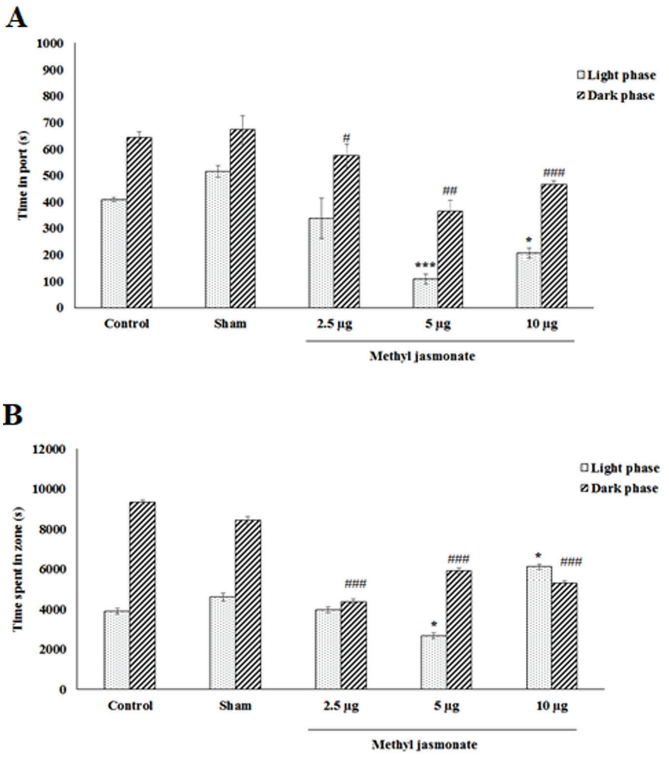

Figure 4. Effect of MJ (2.5, 5, and 10 µg/rat) on the time spent in different ports and zones during light and dark time (n: 7). Data are presented as mean ± SEM.

*p < 0.05, **p < 0.01, ***p < 0.001 *vs* control and sham groups in light phase

#p < 0.05, ###p < 0.001 *vs* control and sham groups in dark phase

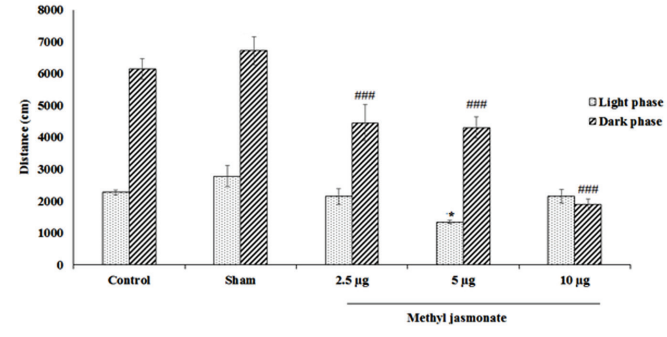

Figure 5. Effect of MJ (2.5, 5, and 10 µg/rat) on the distance traveled by rats in various food zones in light and dark time (n: 7). Data are presented as mean ± SEM

*p < 0.05, ***p < 0.001 *vs* control and sham groups in light phase

###p < 0.001 *vs* control and sham groups in the dark phase

MJ has been shown to have low toxicity in *in vivo* and *in vitro* studies. It showed selective toxicity against tumor cells with no effect on normal human cells.[25] Moreover, MJ (100-300 mg/kg/*i.p.*) treatment could not exert any acute toxic symptoms or death in mice. However, rats treated with MJ at doses of 400 and 500 mg/kg have shown abnormal behavioral changes including ataxia, sedation, and hyperventilation.[26]

MJ, as a linolenic acid-derived cyclopentanone phytohormone, bears structural similarities with prostaglandins.[24] It inhibits prostaglandin E, TNF-α, NFκB-mediated production of nitric oxide, and interleukin LPS -activated murine macrophages.[24,27] Prostaglandin-induced anorexia is associated with alteration of hypothalamic CRF and α-MSH neuronal activities.[28] Arachidonic acid as a prostaglandin precursor has an anorectic effect similar to F2α-induced anorexia in rats.[29] In this study, it is possible to assume that MJ anorexic activity was mediated by manipulation of inflammatory cytokine signaling molecules.

The activities and levels of oxidant/antioxidant agents have been emphasized in studies on metabolic challenges and energy expenders.[30] In this regard, MJ decreased oxidative stress activity in the brain.[9] In addition, it increased reactive oxygen species generation in human cells.[27,31] Furthermore, MJ decreased scopolamine pro-oxidative effects in mice.[9] In a recent study, MJ was able to suppress oxidative stress in rats' hippocampus and prefrontal cortex.[32] Therefore, in this study, it is supposed that MJ antioxidant value might be involved in modulation of feeding behavior of rats.

The localization of Orx1R neurons to the LHA shows their involvement in the central circuitry controlling energy metabolism.[33,34] Here, MJ decreased feeding behavior was associated with Orx1R downregulation in the hypothalamic nuclei including VA and VHC of rats. This indicates that MJ anorexic effects are at least partially medicated with interference on Orx1R signaling in the brain. Orexin neurons are multifunctional neurons that regulate a variety of physiological processes, primarily sleep- and feeding-related behavior.[35,36] Central infusion of an Orx1R agonist increased feeding behavior

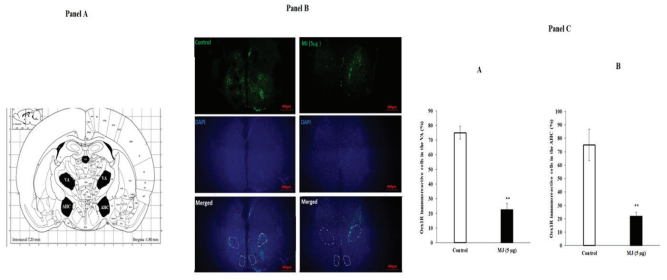

Figure 6. Immunostaining for Orx1R in the hypothalamus of rats. Panel A shows coronal sections through the ventral arterial thalamic nucleus (VA) and anterior hypothalamic arc (AHC) adapted from the atlas of Paxinos and Watson. Panel B indicates Orx1R staining in hypothalmic cells (green), DAPI staining indicates the position of the nuclei in cells (blue), and the merged image of Orx1Rand DAPI of control and MJ (5 µg) groups (n: 4). Panel C, statistical comparison of Orx1R immunoreactive cells in the section from the VA (graph A) and AHC (graph B) of the hypothalamus. Data are presented as mean ± SEM.

**p < 0.01 *vs* control

in rodents and zebrafishes,[37,38] whereas an Ox1R selective antagonist attenuated food intake in rats.[39] Increased food intake and Fos expression in the hypothalamic orexinergic neurons of rats after orexin A administration in the nucleus accumbens. Moreover, food consumption increased in rats treated with orexin.[40] On the other hand, peripheral orexin-A injection did not significantly affect daily food consumption, meal frequency, meal size, and values of total energy expenditure.[36] Notably, it may indicate that administration of orexin A in the central area produces a more powerful effect on increasing food consumption than administration in the peripheral area. Although powerful evidence shows Orx1R involvement in the regulation of feeding behavior, more experiments are still required to elucidate the exact mechanism(s) of MJ interplay with Orx1R neurons to modulate feeding behavior in the brains of rats.

CONCLUSION

Overall, the data indicated that central infusion of phytohormone MJ induced an anorexic effect in rats. Moreover, it decreased Orx1R expression in hypothalamic nuclei. However, more studies are needed to determine the exact mechanism(s) of MJ effects on feeding behavior and Orx1R neuron activity in rats.

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Ethics

Ethics Committee Approval: All the experiments were approved by the Animal Experimentation Ethics Committee of Jiroft University of Medical Sciences, Jiroft, Iran (IR.JMU.REC.1400.001).

Informed Consent: Not necessary.

Peer-review: Externally peer reviewed.

Authorship Contributions

Surgical and Medical Practices: M.Z., M.P., Concept: M.A., Design: R.K., Data Collection or Processing: M.P., R.K., A.A., Analysis or Interpretation: A.A., F.S., Literature Search: M.P., F.S., Writing: R.K., M.A.

Conflict of Interest: No conflict of interest was declared by the authors.

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