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

Short title: Methyl jasmonate effects on feeding behaviors in rats

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Abstract
Objective: The active plant ingredients have been successfully used in modern medicine to control appetites and energy hemostasis. This study designed to evaluate the efficacy of phytohormone methyl jasmonate (MJ) on food-related behaviors in rats.

Methods: The adult male Wistar rats were randomly divided into different groups (7 rats), and infused intracerebroventriculary (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 file like apparatus to assess a 12-hour food-related activities in light and dark times. After behavioral tests, immunofluorescence staining of orexin 1 receptor (Orx1R) was studied in rats’ hypothalamus.

Results: MJ (2.5, 5 and 10 µg/rat) administration significantly decreased food intake in light and dark phases as compared to control group. Moreover, all the MJ -treated groups rats exhibited a decrease in visit to food container in 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 container in light and dark phases in comparison with control rats. Time in zone-related to food, and the locomotor activity were significantly decreased in MJ (5 µg) groups during light time, and in all MJ- injected groups in the dark time. Moreover, hypothalamic expression of Orx1R in rats-treated with MJ (5 µg) significantly lower as compared to control group.

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

Keywords: Methyl jasmonate, Feeding behavior, Ox1R, Rats

INTRODUCTION
Feeding behavior and energy consumed has been considered as an important health concerns in human. In particular, plants-based dietary has been gained scientists’ attentions in last decades. The plant ingredients have been widely used in food industry to control appetite, body weight, metabolic disturbances, and energy intake.

The plant hormone Methyl Jasmonate (MJ), initially isolated from floral scent jasmine plant. The growing bodies of evidence show its value to modulate neurologic process in animals. It structurally is similar to anti-inflammatory prostaglandins (PGE2) which could decrease the induction of interleukin-6 (IL-6), nitric oxide (NO), as well as tumor necrosis factor (TNF-α). It has been shown that MJ attenuates depression-like behavior, anxiolitic responses, and learning and memory decline in rodents. Moreover, MJ was able to decrease stress oxidative indices in mice brain.
In addition, jasmonate usually is ingested with plant nutrients, and may elicit physiologic competence, or toxic effects when ingested at high dosages. It has been used in deity, and the amount of MJ eaten varies, and highly depends on cultural and social feeding behaviors, because content of the compound in plant souses nutrition’s is not the same.

Different areas of the brain the neuromodulators are also intricately involved in energy expungers and food intake. Studies have more emphasized the key role of hypothalamic orexins peptides, orexin-A and orexin B, in appetite and metabolic procedures. These neuropeptides act by the activation of two G-protein-coupled receptors including the orxin 1 receptor (Orx1R), and orxin 2 receptor (Orx2R). Orexin-A is equal at both receptors, however, orexin-B shows more competence to Orx2R. It has been showed that diet intervention is related to changes in OrxRs expression in rat’s brain.

Although MJ is found in the most dietary plant sources, however, there is a lack of study to show the MJ capability to modulate feeding behavior. The research designed to evaluate if central administration of MJ is able to modulate feeding-associated behavior in rats. Moreover, alteration in OrxRs expression in hypothalamus was evaluated in MJ – infused rats.

MATERIAL AND METHODS

Animal

Adult male Wistar rats weighting 230–270 gr 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 with lab environment 30 min in day in cage a week and then valued.

Surgery

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

Drug

Methyl Jasmonate (purity > 95%) was bought from Sigma–Aldrich, and Sodium chloride 0.9% w/v for diluted. Microinjection

The microinjections were accomplished with a Hamilton syringe (1 µl) connected to a needle (27-gauge) via polyethylene tube. Drug infusion was carried out at a rate of 1 µl/min/rat/ side.

Immunofluorescence

The paraffin blocks through the hypothalamic nuclei were sectioned and deparaffinized. The sections were treated for 30 min antigen retrieval by hydrochloric acid solution (2%), for 5 min at room temperature the samples were neutralized by incubating in 0.1 M sodium borate buffer and for 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 for creating a humid environment to prevent tissue drying. After 24 hours, the brain tissue was removed from the refrigerator and washed 4 times with PBS for 5 minutes each time. The secondary antibody was then added at a dilution of 1 to 150 and incubated in a 37 °C incubator for 90 minutes in the dark. The sample was transferred after 3 washes from the incubator to a dark room. It was added DAPI (Sigma-D9542).

Rat preference meter device

Square automated device (60×60 cm) with 30 cm high of a black plexiglass was used. The floor was alienated into nine equal squares. For recording and monitoring of rats’ location as well as food and water consumptions, the apparatus was equipped with underneath load sensors. The animal released from central square (square 5) for assaying preferences behavior. Four middle squares show the preference for the content of the nearest container. Also the corner squares has been considered as for resting animal. Detailed visual cues were also assimilated. The visual cues help the rat to remember the taste memory (Fig. 1).

Experimental design

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

Statistical analysis
All behavioral data were calculated by SPSS software. The data were expressed as means ± standard error of the mean. A two-way ANOVA were applied for analyzed the data. P<0.05 was significant level.

RESULTS
Food consumption
Fig. 2 describes the amount of food intake in different group of rats. Rats-treated with MJ showed significant decrease in food consumption in the both light and dark phases in comparison with control and sham groups. The lowest amount of food consumption in light phase and dark phase were indicated in MJ injected groups at 5 µg and 10 µg, respectively (p<0.001).

Number of visits
There were significant differences between 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 of the control group (Fig 3). In all the groups, entries to food port and zone were significantly increased in dark phase in comparison to light phase (Fig 3).

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

Locomotor activity
The distance traveled in the light phase in MJ –treated group (5 µg) was significantly lower than the control group (p<0.05). In the dark phase, there were increases in travelled 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 were significantly increased as compared with the light phase (p<0.001) (Fig. 5).

IHC
Immunofluorescence staining of Orx1R in hypothalamic nuclei including ventral arterial thalamic nucleus (VA), and anterior hypothalamic arc (AHC) was achieved by help of antibodies directed against Orx1R combined with DAPI nucleus staining. Fig. 6, panel A and B, show 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 Fig. 6, panel C, the numbers of Orx1R positive cells in the hypothalamic nuclei VA (graph A) and AHC (graph B) was significantly attenuated in MJ-treated animals (5 µg) as compared to control group (p<0.01).

DISCUSSION
In data of this study showed that central administration of MJ is able to attenuate feeding-related behaviors in adult male rats. The behavioral effects were accompanying with Orx1R down expression in the hypothalamus of rats. Here, an automated open-field box were used to monitor feeding behavior of rats in a 12 h light and 12 h of darkness cycle \textsuperscript{19}. The acknowledged characters were included the amount of food consumption, time spent, the number of visits, and the distance each rat traveled in ports and zones of food containers \textsuperscript{19}. In line with previous studies on nocturnal animals, the highest nurturing activities were founded in the darkness time \textsuperscript{21}. This study was first to show MJ intervention on feeding behavior in rats. However, previous studies have emphasized MJ efficiency to modulate some of neuronal processes including learning and memory, anxiety-like behavior, stress, and nociception \textsuperscript{8, 22, 23}.

There is little data available showing MJ involvement in feeding behaviors. In rats suffering from arthritis and healthy rat, oral administration of MJ during 18 consecutive days increased the activity of mitochondrial NADP\textsuperscript+ dependent enzymes, and also decreased levels of glucose flux through the glycolysis in the liver. Regarding MJ effects to decreases the hepatic glucokinase activity, and glycolysis it potentially might increase mitochondrial ROS production \textsuperscript{24, 25}.

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

\textsuperscript{19}
MJ as a linolenic acid-derived cyclopentanone phytohormone shows structural similarities with prostaglandins [24]. It inhibits the prostaglandin E, TNF-α, NFκB-mediated production of nitric oxide, and interleukin LPS-activated murine macrophages [24, 27]. It has been indicated that prostaglandin–induced anorexia is associated with alteration of hypothalamic CRF and α-MSH neuronal activities [28]. Arachidonic acid as prostaglandin precursor has been shown anorectic effect similar to F2α-induced anorexia in rats [29]. In this study, it is possible to assume that MJ anorexic activity medicated with manipulation of inflammatory cytokine signaling molecules. The activities, and levels of oxidants/antioxidants agents have been emphasized in studies on the metabolic challenges and energy expenders [30]. In this regards, MJ decreased oxidative stress activity in 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]. So, in this study, it is supposed that MJ anti-oxidant value might be involved in modulation of feeding behavior of rats.

The localization of Orx1R neurons to the LHA, show they involvement in central circuitry controlling of energy metabolism [33, 34]. Here, MJ- decreased feeding behavior was associated with Orx1R down expression in the hypothalamic nuclei including VA, and VHC of rats. It indicates that MJ anorexic effects at least partially mediated with interference on Orx1R signaling in the brain. Orexin neurons are multifunctional neurons that regulate verity of physiological processes primarily sleep and feeding-related behavior [35, 30]. Central infusion of Orx1R agonist increased feeding behavior in rodents and zebrafishes [37, 38]. Whereas, Orx1R selective antagonist attenuated food intake in rats [39]. Increased food intake and Fos expression in hypothalamic orexigenic neurons of rats after orexin A administration in the nucleus accumbens. Moreover, food consumption has been increased in rats treated with orexins [40]. On the other hands, 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 centraler area produces a more powerful effect on heighten food consumption than in administration in peripheral. Although, powerful evidences show Orx1R involvement in regulation of feeding behavior, however, more experiments are still required to elucidate the exact mechanism(s) of MJ interplay with Orx1R neurons to modulate feeding behavior in rats’ brain.

CONCLUSION

Overall, the data indicated 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 neurons activity in rats.

CONFLICT OF INTEREST

None

References


Figures

Fig. 1. Overlook of rat Preference meter apparatus.
Fig. 2. Effect of MJ (2.5, 5, and 10 µg/rat) on rats’ food consumption (in grams) in light time 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 dark phase
Fig. 3. Effect of MJ (2.5, 5, and 10 µg/rat) on the number of visits to different ports (a) and zone (b) in light time and dark time (n=7). Data are presented as mean ±SEM. *p<0.05, **p<0.01 vs control and same groups, ***p<0.001 vs control and same groups, ###p<0.001 vs control and same groups.
Fig. 4. Effect of MJ (2.5, 5, and 10 µg/rat) on the time spent in different ports and zones in light time 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

Fig. 5. Effect of MJ (2.5, 5, and 10 µg/rat) on rats’ distance traveled in various food zones in light time 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 dark phase

Fig. 6. Immunostaining for Orx1R in hypothalamus of rats. Panel A shows coronal sections through ventral arterial thalamic nucleus (VA), and anterior hypothalamic arc (AHC) adapted from the atlas of Paxinos and Watson. Panel B indicates Orx1R staining in the hypothalamus cells (green), DAPI staining indicates the position of the nuclei in cells (blue) and the merged image of Orx1R, and 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 hypothalamus. Data are represented as mean ± SEM. **p < 0.01 vs control