Neuroscience Letters 631 (2016) 104–108

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Research article

mGluR1/5 activation in the lateral hypothalamus increases food intake via the endocannabinoid system☆,☆☆

Asai Sánchez-Fuentes[a], Bruno A. Marichal-Cancino[a], Mónica Méndez-Díaz[a], Alline L. Becerril-Meléndez[a], Alejandra E. Ruiz-Contreras[b], Oscar Prospéro-Garcia[a,*]

[a] Grupo de Neurociencias, Laboratorio de Cannabinoides, Departamento de Fisiología, Facultad de Medicina, Universidad Nacional Autónoma de México, Mexico

[b] Lab. Neurogenomica Cognitiva, Coord. Psicobiología y Neurociencias, Fac. Psicologia, Universidad Nacional Autónoma de México, México D.F., Mexico

HIGHLIGHTS

- Suppression of diacylglycerol lipase in the lateral hypothalamus prevented the increment in food intake induced by CHPG (mGluR1/5 agonist).
- Blockade of CB_1 receptors (with AM251) in the lateral hypothalamus prevented the increment in food intake induced by CHPG (mGluR1/5 agonist).
- Lateral hypothalamic activation of mGluR1/5 increase food intake via endocannabinoid system.

ARTICLE INFO

Article history:
Received 25 May 2016
Received in revised form 14 July 2016
Accepted 13 August 2016
Available online 16 August 2016

Keywords:
2-AG
Food intake-lateral hypothalamus
mGluR1/5
Endocannabinoid system

ABSTRACT

Mounting evidence has shown that glutamatergic and endocannabinoid systems in the hypothalamus regulate mammalian food intake. Stimulation of hypothalamic mGluR1/5 and CB_1 receptors induces hyperphagia suggesting a possible interaction between these systems to control food intake. In addition, synthesis of endocannabinoids has been reported after mGluR1/5 stimulation in the brain. The aim of this study was to examine the potential cannabinergic activity in the food intake induction by lateral hypothalamic stimulation of mGluR1/5. Wistar albino male rats received bilateral infusions in the lateral hypothalamus (LH) of: (i) vehicle; (ii) (RS)-2-Chloro-5-hidroxyphenylglycine (CHPG; mGluR1/5 agonist); (iii) 2-AG (CB_1 endogenous agonist); (iv) AM251 (CB_1 antagonist); (v) tetrahydrolipstatin (THL, 1.2 μg; diacyl-glycerol lipase inhibitor); and (vi) combinations of CHPG + with the other aforementioned drugs. Food intake was evaluated the first two hours after drug administration. CHPG significantly increased food intake; whereas CHPG in combination with a dose of 2-AG (with no effects on food intake) greatly increased food ingestion compared to CHPG alone. The increase induced by CHPG in food intake was prevented with AM251 or THL. These results suggest that activation of mGluR1/5 in the lateral hypothalamus induces an orexigenic effect via activation of the endocannabinoid system.

1. Introduction

Energy balance is crucial for maintaining life and its associated activities. This process is of such importance that it requires the participation of several systems and mechanisms at all levels (i.e., cellular, tissular, systemic, etc.) [1]. Among central systems, there is extensive literature supporting the role of the lateral hypothalamus (LH) in the integration of signals conveying information about the energy status of the body [2,3]. For example, deficit or excess in circulating glucose levels immediately enhance or inhibit (via the lateral hypothalamus), respectively, the motivation to seek and ingest food [1–4]. The glutamatergic activation of the LH also promotes food seeking and its ingestion [5,6]. These effects seem to be mediated by the metabotropic glutamate receptor 1/5 (mGluR1/5)

☆ Web page: http://www.facmed.unam.mx/deptos/fisiologia/investigadores/prospero_g.html

☆☆ This work was supported by the Dirección General de Asuntos del Personal Académico-Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica, Universidad Nacional Autónoma de México (DGAPA-PAPIIT-UNAM), Grant number IN224314 to OPG and CONACyT 176196 to AERC. We declare no conflict of interest.

* Corresponding author at: Departamento de Fisiología, Facultad de Medicina, Universidad Nacional Autónoma de México, Apdo. Postal 70–250, México D.F. 04510, Mexico.
E-mail address: opg@unam.mx (O. Prospéro-Garcia).

http://dx.doi.org/10.1016/j.neulet.2016.08.020
0304-3940/

[7]. For example: infusion of CHPG (mGluR1/5 agonist) into the LH, induces an increase in food intake in rats [8]. Interestingly, the induction of food intake by CHPG into the LH was detected only during the first two hours after the infusion [8] suggesting a short-lasting course of action.

On the other hand, other molecules which physiologically modulate hypothalamic control of food intake and energy balance are the endocannabinoids [9–13]. They are lipid derived molecules which interact with other G-protein coupled receptors/transient receptor potential channels (e.g., GPR55, GPR118, GPR119, TRPV1, 5-HT3, etc.) [14–18], in addition to cannabinoid receptors type 1 and type 2 (CB_1 and CB_2, respectively) [14]. Interestingly, activation of mGluR1/5 stimulates diacylglycerol lipase type α (DGL-α), which in turn, leads to the biosynthesis of endocannabinoid arachidonic acid derivate 2-arachidonyl-sn-glycerol (2-AG) [19–21]. The synthesis of 2-AG after mGluR1/5 stimulation has previously been reported in the periaqueductal gray matter modulating antinociception [22].

Considering this background, our hypothesis was that interaction between mGluR1/5 and the endocannabinoid system may exist in the hypothalamus (whose activity is under glutamatergic and cannabinergic control). Hence, this study investigated whether the endocannabinoid system may be involved in the previously reported [8] orexigenic effect induced during the first 2 h after activation of mGluR1/5 in the LH. For this purpose, two experiments were carried out. Experiment 1 was designed using a minimal number of animals in a Latin-square design to test the potential existence of a relationship between glutamatergic and cannabinergic systems in the modulation of food intake. Once we probed the potential relationship between systems, experiment 2 was designed using independent groups (because the number of treatments make the Latin-square design difficult) to further analyze the pharmacological profile of this relationship.

2. Methods

2.1. Subjects

Wistar albino male rats (weight 250–300 g at the beginning of experiments; n = 54) were used. Animals were housed individually in a temperature controlled environment (21 ± 1 °C and 52% humidity)with a 12-h light/dark cycle (light on at 20:00 h) and ad libitum access to water and food (Rodent Laboratory Chow, 3.3 cal/g; protein: fat: carbohydrates 28.5:13.5:58) before and throughout the experiments. Rats were handled according to the Public Health Service (PHS) Policy of Humane Care and Use of Laboratory Animals and the Ethics Committee guidelines of the School of Medicine (NOM-062-ZOO-1999), Universidad Nacional Autónoma de México (UNAM).

2.2. Surgery

Underanesthesia (ketamine 66 mg/kg + xylazine 0.26 mg/kg + acepromazine 1.3 mg/kg + saline; i.p.), rats were bilaterally implanted with a couple of guide cannulae (23 gauge) in the LH (P = −2.6, L = ± 1.8, V = −7; Fig. 1) [23] and affixed to the skull with dental cement. According with the Charles et al. [8] report, animals were left for 10 days after surgery for recovery. When experiments ended, brains were prepared for histological analysis with cresyl violet staining to verify the correct placement of the injector (Fig. 1). Three animals were discarded from analysis because the injector was misplaced.

2.3. Drugs

The compounds used in the experiments were: agonist to mGluR1/5 (RS)-2-Chloro-5-hidroxyphenylglycine sodium salt (CHPG, Tocris Bioscience), inverse agonist of CB_1 receptor AM251 (Sigma–Aldrich), DGL inhibitor tetrahydrolipstatin (THL; Sigma–Aldrich) and the endocannabinoid 2-AG (Tocris Bioscience). Dimethyl sulfoxide 100% (DMSO) was use as a solvent. The concentration used of CHPG (5.6 μg) was equimolar to that used by Charles et al. [8]; whereas that for THL (1.2 μg) and AM251 (1.4 μg) were selected from previous results from our group [23–25]. 2-AG (0.1 μg) was used based on its affinity profile [26].

The final volume for all injections was 0.5 μl per side, injected at a rate of 0.1 μl/min. Administration of drugs was performed by mean of an infusion pump (kdScientific®, USA) and 30-gauge injectors, which protruded 1 mm from the end of the guide cannula.

2.4. Procedures

For experiments 1 and 2, rats were initially weighed; subsequently, they were randomly and bilaterally infused in the LH (at the beginning of dark phase of the cycle at 8:00 h). They were put in their cage with pre-weighed food and the leftover food was recorded after 120 min. The food intake evaluation for 24 h did not show differences among groups (data not shown). To ensure measures included food intake only, food spillage was collected. As we did not video-tape rats' behaviour, additional behaviour induced by these treatments was not evaluated.

2.5. Experiment 1

A Latin-square design was used for the following treatments (n = 9): vehicle (DMSO 100%), CHPG (5.6 μg), THL (1.2 μg) and the combination of CHPG (5.6 μg) + THL (1.2 μg). Considering that the effect of CHPG was not detected after 24 h [8], we established an interval of 48 h between treatments and the experiment ended when each animal received all treatments. Food intake was recorded as described above.

2.6. Experiment 2

Six independent groups of rats (n = 7) received bilateral infusions into the LH of one of the following treatments: vehicle, CHPG, 2-AG, AM251, 2-AG + CHPG, and AM251 + CHPG. As described above, once rats were injected they were put back in their cages. Food amount ingested was evaluated as previously described.

2.7. Statistical analyses

Although our rats were exhibiting a quite similar weight, to be more precise as to the g of food they ingested in relation to their weight, the amount of food consumed in both experiments is reported as an index, according to the following formula: [(g of food eaten/body weight) × 100]. Data are expressed as mean ± SEM of food intake index. Data analyses were carried out using a one way repeated measures analysis of variance (RM-ANOVA) for experiment 1 and one way analysis of variance(ANOVA) for experiment 2. A Student-Newman-Keuls test was used as a *post hoc* test. A standard significance level of $P < 0.05$ was used for all tests.

3. Results

3.1. Experiment 1

Table 1 shows the body weight of different groups, food intake in grams and food intake index values. The one-way ANOVA