Aqueous Extract of *Ma huang* Decreases Neuropeptide Y Expression in the Hypothalamus of Rats

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Ma huang, the dried plant stem of Ephedra Intermedia Schrenk et C.A., is one of the well known medicinal herbs, and has been used for the diaphoretic, antiasthmatic, and diuretic actions. *Ma huang* is an ephedrine type alkaloid used for the weight loss and energy expenditure. Medications based on the *Ma huang* have been found to be effective in the treatment of obesity. Neuropeptide Y (NPY), a 36-amino-acid peptide and concentrated in the hypothalamus, stimulates feeding desire and decrease energy expenditure. In the present study, the effect of *Ma huang* on the expression of NPY in the rat hypothalamus was investigated using immunohistochemistry. The present results demonstrated that *Ma huang* treatment suppressed weight gain and NPY expression in the hypothalamus depending upon the dosage used. Based on the results, it can be suggested that *Ma huang* treatment is effective in curbing the desire for food via modulation of NPY expression under the normal conditions.

Key words : *Ma huang*, neuropeptide Y, hypothalamus, immunohistochemistry, weight loss

Introduction

Ma huang, the dried plant stem of Ephedra Intermedia Schrenk et C.A., is one of the well known medicinal herbs for the diaphoretic, antiasthmatic, and diuretic actions. Ma huang contains approximately 1.25% ephedrine as well as several other related alkaloids such as pseudoephedrine, methylephedrine, and norpseudoephedrine[1]. The stimulating and sympathomimetic effects of ephedrine are mediated by its agonistic effects on the α1, β1, and β2 receptors[2]. The sympathomimetic agonists acting on both the α- and β -adrenergic receptors lead to increased cardiac rate and contractility, peripheral vasoconstriction, bronchodilation, and stimulation of central nervous system (CNS)[3,4]. *Ma huang* is an ephedrine type alkaloid and has been used for weight loss and energy expenditure[5-8]. Medications based on the *Ma huang* have been found to be effective for the treatment of obesity[9]. Hypothalamus is an important area of the brain for the regulation of food intake and energy balance[10]. Neuropeptide Y (NPY) is a 36-amino-acid peptide and concentrated in the hypothalamus, and stimulates feeding and decreases energy expenditure[11]. It is notably one of the most abundant brain peptides in the paraventricular nucleus (PVN) and arcuate nucleus (ARN) and other regions implicated in the regulation of feeding behavior, energy balance, and pituitary secretion[12]. In the present study, the effect of *Ma huang* on the hypothalamic NPY expression was investigated using immunohistochemistry.

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Materials and Methods

1. Animals and treatments

Male Sprague-Dawley rats weighing 250 ± 10 g (8 weeks old) were used for the experiment. Each animal was housed at a controlled temperature (20 ± 2 ℃) and was maintained in light-dark cycles, each cycle consisting of 12 h of light and 12 h of darkness (lights on from 07:00 h to 19:00 h) with food and water available ad libitum. The experimental procedures were performed in accordance with the animal care guidelines of National Institute of Health (NIH) and Korean Academy of Medical Sciences. Animals were divided into five groups: the control group, the 10 mg/kg *Ma huang*-treated group, the 50 mg/kg *Ma huang*-treated group, the 100 mg/kg *Ma huang*-treated group, and the 200 mg/kg *Ma huang*-treated group.

2. Preparation of aqueous extract of *Ma huang*

Ma huang used in this study was obtained from Kyung-Dong market (Seoul, Korea). After washing, *Ma huang*

was immersed in cold water for 12 h. To obtain an aqueous extract of *Ma huang*, 300 g of *Ma huang* was added to distilled water, heat extracted, concentrated with a rotary evaporator, and lyophilized. The resulting powder, weighing 32.2 g, was diluted with saline solution. After filtering through a 0.45 mm syringe filter, *Ma huang* was administered intraperitoneally once a day for 3 consecutive days at the respective doses of 10, 50, 100, and 200 mg/kg of body weight.

3. Tissue preparation

At the beginning of the sacrificial procedure, animals were weighed and overdosed with Zoletil 50® (10 mg/kg, i.p.; Vibac Laboratories, Carros, France). After a complete lack of response was observed, the rats were transcardially perfused with 50 mM phosphate-buffered saline (PBS) and then with 4 % paraformaldehyde in 100 mM phosphate buffer (PB) at pH 7.4. The brains were dissected, postfixed in the same fixative overnight, and transferred into a 30 % sucrose solution for cryoprotection. Serial coronal sections of 40 ㎛ thickness were made using a freezing microtome (Leica, Nussloch, Germany).

4. NPY immunohistochemistry

Average eight sections were collected from each brain for immunohistochemistry. Free-floating tissue sections were washed twice for 15 min in 50 mM PBS, and then permeabilized in 0.2 % Triton X-100 for 30 min. After washing twice with PBS, sections were incubated overnight with rabbit anti-NPY antiserum (DiaSorin, Stillwater, MN, USA) at a dilution of 1:4000. Sections were washed twice in PBS and incubated for 1 h with biotinylated anti-rabbit antibody. Bound secondary antibody was then amplified with the Vector Elite ABC kit (Vecta Laboratories, Burlingame, CA, USA). The antibody-biotin-avidin-peroxidase complexes were visualized using 0.05% diaminobenzidine. The intensities of NPY-specific staining were assessed in a quantitative fashion according to a microdensitometrical method based on optical density (mean gray scale)[13] using an image analyzer (Media Cybernetics Inc., Silver Spring, MD, USA). Before starting the image analysis, the light source was adjusted to the brightness generating the best possible contrast between positive- and negative-staining cells.

5. Data analysis

Statistical significance of differences was determined by one-way analysis of variance (ANOVA) followed by Duncan's post-hoc analysis, and results were expressed as mean ± standard error mean (S.E.M.). Differences were considered significant for $P < 0.05$.

Results

1. Effect of *Ma huang* on body weight change

At the 3 days after commencement of experiment, the body weight was changed from 258.87 ± 2.60 to 295.00 ± 2.88 g in the control group, from 251.87 ± 4.58 to 281.25 ± 1.25 g in the 10 mg/kg Ma huang-treated group, from 258.00 ± 3.58 to 268.00 ± 4.89 g in the 50 mg/kg *Ma huang*-treated group, from 251.70 ± 5.57 to 249.00 ± 5.09 g in the 100 mg/kg *Ma huang*-treated group, and from 255.60 ± 2.51 to 239.00 ± 2.44 g in the 200 mg/kg *Ma huang*-treated group. In the present results, *Ma huang* treatment was shown to suppress body weight gain in a dose-dependent manner (Fig. 1).

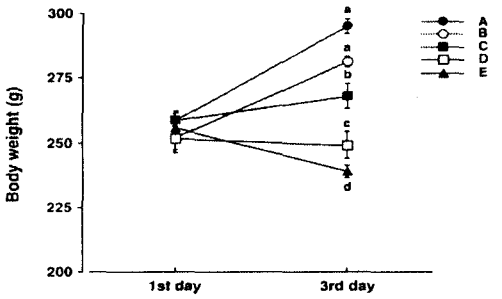

Fig. 1. Effect of *Ma huang* on weight change in each group. (A) Control group; (B) 10 mg/kg *Ma huang*-treated group; (C) 50 mg/kg *Ma huang*-treated group; (D) 100 mg/kg *Ma huang*-treated group; (E) 200 mg/kg *Ma huang*-treated group.

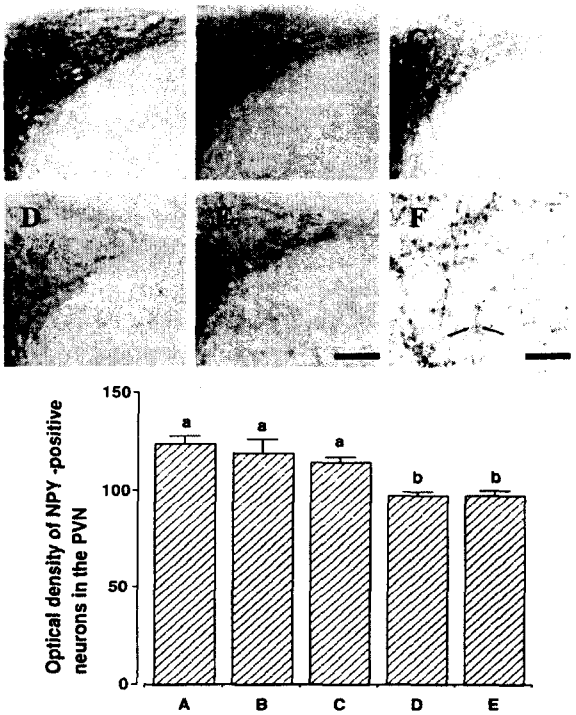

Fig. 2. Effect of *Ma huang* on the expression of neuropeptide Y(NPY) in the paraventricular nucleus (PVN) in each group. (A) Control group; (B) 10 mg/kg *Ma huang*-treated group; (C) 50 mg/kg *Ma huang*-treated group; (D) 100 mg/kg *Ma huang*-treated group; (E) 200 mg/kg *Ma huang*-treated group. Above: Photomicrographs of NPY expression in the PVN in each group. Scale bar represents 100 ㎛ (A - E) and 25 ㎛ (F). Below: Mean optical density of NPY in the PVN.

2. Effect of *Ma huang* on NPY expression in the PVN

The intensity of NPY immunoreactivity in the PVN of the hypothalamus was 123.93 ± 4.16 in the control group, 118.88 ± 7.15 in the 10 mg/kg *Ma huang*-treated group, 114.20 ± 2.75 in the 50 mg/kg *Ma huang*-treated group, 94.52 ± 2.17 in the 100 mg/kg *Ma huang*-treated group, and 97.20 ± 2.79 in the 200 mg/kg *Ma huang*-treated group. In the present results, *Ma huang* treatment was shown to suppress NPY expressions in the PVN in a dose-dependent manner (Fig. 2).

3. Effect of *Ma huang* on NPY expression in the ARN

The intensity of NPY immunoreactivity in the ARN of the hypothalamus was 140.75 ± 0.94 in the control group, 144.89 ± 1.87 in the 10 mg/kg *Ma huang*-treated group, 142.47 ± 1.62 in the 50 mg/kg *Ma huang*-treated group, 132.87 ± 4.04 in the 100 mg/kg *Ma huang*-treated group, and 129.03 ± 1.97 in the 200 mg/kg *Ma huang*-treated group. In the present results, *Ma huang* treatment was shown to suppress NPY expressions in the PVN in a dose-dependent manner (Fig. 3).

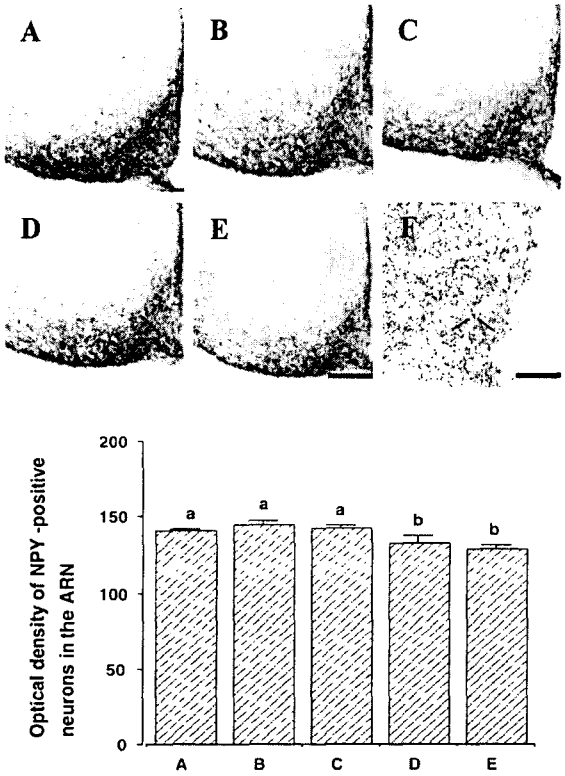

Fig. 3. Effect of *Ma huang* on the expression of neuropeptide Y (NPY) in the arcuate nucleus (ARN) in each group. (A) control group; (B) 10 mg/kg *Ma huang*-treated group; (C) 50 mg/kg *Ma huang*-treated group; (D) 100 mg/kg *Ma huang*-treated group; (E) 200 mg/kg *Ma huang*-treated group. Above: Photomicrographs of NPY expression in the ARN in each group. Scale bar represents 100 ㎛ (A - E) and 25 ㎛ (F). Below: Mean optical density of NPY in the ARN.

Discussion

In the present study, significant weight loss was observed in the *Ma huang*-treated groups. The plant genus Ephedra, commonly known also *Ma huang*, is a botanical source of ephedrine alkaloids has been used as a "natural stimulant" or for thermogenic diet aid[14]. Body weight of the over-weighted men and women was shown to be reduced by administration of *Ma huang* and caffeine mixtures[15]. NPY is one of the most important signals in the hypothalamic neural circuitry that regulates food intake and body weight[16,17]. It has been indicated that hypothalamic NPY plays an important role in regulation of appetite in mammals[18,19]. In the present results, NPY expression in the PVN and ARN was significantly decreased by administration of the aqueous extract of Ma huang as a dose-dependent manner. The various subregions of the hypothalamus play important roles in the regulation of food intake and energy expenditure. Of these, ARN is composed with elongated neuronal cell bodies occupying nearly one-half of the hypothalamus and apparently subdivided into several functional domains[20], and is situated around the base of the third ventricle immediately above the median eminence. NPY, a potent stimulator of food intake, is co-localized in a population of neurons in the ARN[21]. ARN has extensive reciprocal connections with other hypothalamic regions including PVN. PVN lies beside the top of the third ventricle in the anterior hypothalamus. It is an integrating center which converges various neural pathways that influence energy homeostasis. PVN is richly supplied by axons projecting from neurons in the ARN. It contains abundant appetite-modifying neurotransmitters, including NPY, in its terminals, and is particularly sensitive to these neurotransmitters[20,22].

The present results demonstrated that Ma huang treatment suppresses weight gain and inhibits NPY expression in PVN and ARN of the hypothalamus as dose-dependently. Based on the results, it can be suggested that *Ma huang* treatment is effective in curbing the desire for food via modulation of NPY expression in the normal conditions.

Acknowledgments

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