Aqueous Extract of *Ma huang* Suppresses Neuropeptide Y Expression in Food-Deprived Rat Hypothalamus

Ee-Hwa Kim  
*Department of Meridian and Acupoint, College of Oriental Medicine*  
*Semyung University, Jechon, Korea*

Mal-Soon Shin, Hyun-Kyung Chang, Taeck-Hyun Lee, Mi-Hyeon Jang, Min-Chul Shin,  
Sam-Jun Lee and Chang Ju Kim  
*Department of Physiology, College of Medicine*  
*Kyung Hee University, Seoul, Korea*

Abstract: *Ma huang*, the dried plant stem of *Ephedra Intermedia* Schrenk et C.A., contains an ephedrine-type alkaloid and has been used for weight loss. Neuropeptide Y (NPY), a 36-amino acid peptide, is concentrated in the hypothalamus and stimulates feeding desire. In this study, the effect of *Ma huang* on the expressions of NPY in the hypothalamus of rats was investigated using immunohistochemistry. Food-deprivation enhanced the NPY expression in the hypothalamus. *Ma huang* suppressed the food-deprivation-induced enhancement of NPY expression. Present results suggest that *Ma huang* curbs the food desire by suppressing the NPY expression under food-deprivation conditions.

*Keywords*: *Ma huang*; Neuropeptide Y; Hypothalamus; Immunohistochemistry.

Introduction

*Ma huang*, the dried plant stem of *Ephedra Intermedia* Schrenk et C.A., is a well known medicinal herb. *Ma huang* has diaphoretic, anti-asthmatic and diuretic properties (Reinhard *et al.*, 1991; Yuan *et al.*, 1998). *Ma huang* contains approximately 1.25% ephedrine as well as several other related alkaloids such as pseudoephedrine, methylephedrine and
norpseudoephedrine (White et al., 1997). The stimulatory and sympathomimetic effect of ephedrine is mediated by its agonistic effect on the α1-, β1- and β2-adrenergic receptors (Haller and Benowitz, 1997), resulting in increase in the cardiac rate and the contractility, peripheral vasoconstriction, bronchodilation and the stimulation of the central nervous system (CNS) (Fouad-Tarazi et al., 1995; Walker et al., 1998).

The hypothalamus is an important area of the brain for the regulation of food intake and energy balance (Dryden et al., 1997). Many neuropeptides are implicated in the control of food intake and energy expenditure. Of particular interest is neuropeptide Y (NPY) that induces a powerful feeding response following central administration (Stanley et al., 1985). NPY is a 36-amino acid peptide, which acts as a key neurotransmitter in the regulation of food intake. It is notably one of the most abundant brain peptides in the paraventricular nucleus (PVN) and the arcuate nucleus (ARN) and other regions implicated in the regulation of feeding behavior, energy balance and pituitary secretion (Chronwall et al., 1985).

The ARN is extensively connected to other hypothalamic regions such as the PVN, dorsomedial hypothalamic nucleus (DMH), ventromedial hypothalamic nucleus (VMH) and lateral hypothalamus (Broberger et al., 1998; Hahn et al., 1998). The PVN is rich in the axons projecting from the ARN-NPY/Agouti-Related Protein (AGRP), pro-opiomelanocortin (POMC)/cocaine-amphetamine regulated transcript (CART) neurons, and the orexin neurons of the lateral hypothalamus (Elmquist et al., 1999). Within the hypothalamus, NPY is synthesized in the ARN at the base of the third ventricle that project through the LHA to the PVN and DMH. The NPYergic neurons that project to the end of the PVN, DMH and other appetite-regulating areas appear to be particularly important in the control of feeding behavior (Bai et al., 1985; Chronwall et al., 1985; Inui, 1999; Stanley et al., 1992).

The increase in the biosynthesis as well as the release of NPY in the discrete neuronal pathways constitutes the specific hypothalamic response to starvation (Brady et al., 1990). *Ma huang* contains an ephedrine-type alkaloid and has been used for weight loss and energy expenditure (Astrup et al., 1992; Zaacks et al., 1999). Medications derived from *Ma huang* have been shown to be effective in the treatment of obesity (Boozer et al., 2001). In the present study, the effect of *Ma huang* on the expression of NPY in the ARN and PVN following food-deprivation was investigated via immunohistochemistry.

Materials and Methods

Animals and Treatments

Male Sprague-Dawley rats weighing 230 ± 10 g (eight weeks old) were used for the experiment. Animals were housed in a room with controlled temperature (20 ± 2°C) and light-dark cycle consisting 12 hours light and 12 hours darkness (lights on from 07:00 hour to 19:00 hour). Experiments were performed in accordance to the animal care guidelines of the National Institute of Health (NIH) and Korean Academy of Medical Sciences.

Animals were randomly divided into six groups: the fed (control) group, the food-deprived group, the food-deprived and 10 mg/kg *Ma huang*-treated group, the food-deprived and...
50 mg/kg Ma huang-treated group, the food-deprived and 100 mg/kg Ma huang-treated group, and the food-deprived and 200 mg/kg Ma huang-treated group (n = 5 in each group). Animals of the fed group received abundant food and water, while food was withheld from those of the food-deprived groups for 72 hours prior to experiments.

Preparation of Aqueous Extract of Ma huang

Ma huang was obtained from Kyung-Dong market (Seoul, Korea). After washing, Ma huang was immersed in cold water for 12 hours. 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 dissolved in saline and sterilized by filtering through a 0.45 µm syringe filter. Ma huang thus prepared was administered intraperitonally to rats once a day for three consecutive days at the doses of 10, 50, 100 and 200 mg/kg body weight. Control rats were treated with saline once a day for three days.

Tissue Preparation

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 µm thickness were made using a freezing microtome (Leica, Nussloch, Germany).

NPY Immunohistochemistry

An average eight sections were collected from the brain for immunohistochemistry. Free-floating tissue sections were washed twice for 15 minutes in 50 mM PBS, and then permeabilized in 0.2% Triton X-100 for 30 minutes. 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 hour with biotinylated anti-rabbit antibody. Bound secondary antibody was then amplified with the Vector Elite ABC kit (Vector Laboratories, Burlingame, CA, USA). The antibody-biotin-avidin-peroxidase complexes were visualized using 0.05% diaminobenzidine. The intensity of NPY-specific staining was assessed in a quantitative fashion according to a microdensitometrical method based on optical density (mean gray scale) (Jang et al., 2002) 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.
Data Analysis

Statistical significance of differences was determined by one-way analysis of variance (ANOVA) followed by Duncan’s post-hoc analysis. The data were presented as the mean ± standard error mean (SEM). Difference was considered significant at \( p < 0.05 \).

Results

The Effect of Ma huang on the Change in Body Weight

Three days after the initiation of experiments, the body weight of fed (control) animals was changed from 233.00 ± 4.43 g to 239.50 ± 4.11 g, the food-deprived group from 232.62 ± 5.20 g to 199.25 ± 4.88 g, the food-deprived and 10 mg/kg Ma huang-treated group from 234.50 ± 3.12 g to 203.00 ± 2.79 g, the food-deprived and 50 mg/kg Ma huang-treated group from 231.62 ± 3.65 g to 200.00 ± 2.54 g, the food-deprived and 100 mg/kg Ma huang-treated group from 232.37 ± 3.35 g to 203.50 ± 1.70 g, and the food-deprived and 200 mg/kg Ma huang-treated group from 229.62 g ± 2.42 to 193.75 ± 1.43 g. Food-deprivation for three days suppressed weight gain and food-deprived and Ma huang-treated groups also showed weight loss. The difference was not statistically significant (Fig. 1).

The NPY Expression in the PVN

The intensity of NPY immunoreactivity in the PVN of the hypothalamus was 132.34 ± 3.34 g in the fed (control) group, 149.46 ± 0.89 g in the food-deprived group, 132.26 ± 1.27 g in the food-deprived and 10 mg/kg Ma huang-treated group, 127.47 ± 3.36 g in the food-deprived and 100 mg/kg Ma huang-treated group, and 127.47 ± 3.36 g in the food-deprived and 200 mg/kg Ma huang-treated group.

Figure 1. The effect of Ma huang on the body weight of rats. (A) fed group, (B) food-deprived group, (C) food-deprived and 10 mg/kg Ma huang-treated group, (D) food-deprived and 50 mg/kg Ma huang-treated group, (E) food-deprived and 100 mg/kg Ma huang-treated group, and (F) food-deprived and 200 mg/kg Ma huang-treated group. * Indicates \( p < 0.05 \) compared to fed group. # Indicates \( p < 0.05 \) compared to 1st day of the experiments.
food-deprived and 50 mg/kg Ma huang-treated group, 133.50 ± 1.82 g in the food-deprived and 100 mg/kg Ma huang-treated group, and 130.35 ± 2.83 g in the food-deprived and 200 mg/kg Ma huang-treated group.

In the present results, the NPY expression in the PVN was enhanced in the food-deprived rats and the treatment with Ma huang extract suppressed the food-deprivation-induced increase of NPY expression. The differences among the Ma huang-treated groups, however, were not statistically significant (Fig. 2).

The NPY Expression in the ARN

The intensity of NPY immunoreactivity in the ARN of the hypothalamus was 132.74 ± 6.56 g in the fed (control) group, 156.92 ± 1.95 g in the food-deprived group, 141.98 ± 1.39 g in the 10 mg/kg Ma huang-treated group, 147.34 ± 1.00 g in the food-deprived and

Figure 2. The effect of Ma huang on the expression of neuropeptide Y (NPY) in the paraventricular nucleus (PVN). (A) fed group, (B) food-deprived group, (C) food-deprived and 10 mg/kg Ma huang-treated group, (D) food-deprived and 50 mg/kg Ma huang-treated group, (E) food-deprived and 100 mg/kg Ma huang-treated group, and (F) food-deprived and 200 mg/kg Ma huang-treated group. Above: Photomicrographs of NPY expression in the PVN. A scale bar represents 100 µm. Below: The mean optical density of NPY in the PVN. * Represents p < 0.05 compared to the fed group. # Represents p < 0.05 compared to the food-deprived group.
In the present results, the NPY expression in the ARN was enhanced in the food-deprived rats and the treatment with Ma huang extract suppressed the food-deprivation-induced increase of NPY expression. The differences among the Ma huang-treated groups, however, were not statistically significant (Fig. 3).

Discussion

The plant genus Ephedra, commonly known as Ma huang, is a botanical source of ephedrine alkaloids used as a natural stimulant or thermogenic diet aid (Marwick, 1995). Ma huang, in combination with caffeine, has been reported to reduce body weight in overweight men and women (Boozer et al., 2002).

50 mg/kg Ma huang-treated group, 148.54 ± 1.38 g in the food-deprived and 100 mg/kg Ma huang-treated group, and 146.89 ± 1.41 g in the food-deprived and 200 mg/kg Ma huang-treated group.

In the present results, the NPY expression in the ARN was enhanced in the food-deprived rats and the treatment with Ma huang extract suppressed the food-deprivation-induced increase of NPY expression. The differences among the Ma huang-treated groups, however, were not statistically significant (Fig. 3).
NPY is one of the most important neurotransmitters in the hypothalamic neural circuitry that regulates food intake and body weight (Leibowitz, 1990). Hypothalamic NPY plays an important role in the regulation of the appetite in mammals (Marks et al., 1993). Various subregions of the hypothalamus are implicated in the regulation of food intake and energy expenditure.

The ARN is composed of elongated neuronal cell bodies that occupy nearly one-half of the hypothalamus and subdivided into several functional domains (Williams et al., 2001). The ARN is located at the base of the third ventricle immediately above the median eminence. NPY, a potent stimulator of food intake, is co-localized with the neurons in the ARN (Hahn et al., 1998). The ARN is extensively connected to other hypothalamic regions including the PVN.

The PVN lies on the side of the top of the third ventricle in the anterior hypothalamus. The PVN is an integrating center where various neural pathways that influence energy homeostasis converge. The PVN is rich in the axons projecting from the neurons in the ARN. The PVN contains abundant appetite-modifying neurotransmitters including NPY in its terminals and is particularly sensitive to these neurotransmitters (Swanson and Sawchenko, 1983; Williams et al., 2001).

In the present study, NPY expression in the PVN and ARN was enhanced in the food-deprived rats. These results are consistent with previous reports: the enhanced expression of NPY mRNA was observed in the PVN and ARN under food-deprivation conditions (Beck et al., 1990). Our data showed that the elevated level of NPY expression in the PVN and ARN of the food-deprived rats was significantly suppressed by Ma huang treatment. Ma huang mediated the suppressive effect dose-independently.

Ma huang at all doses tested suppressed the expression of NPY equally efficiently: Ma huang at doses higher than 10 mg/kg showed the “saturation effect” on suppressing the NPY expression. Herbal medicines sometimes show the dose-independent pharmacological effect since they contain several constituents (Makino et al., 2001). The pharmacological effect of an herb is the sum of all components. It is possible that the dose-independent fashion of the effect of Ma huang on the NPY expression may be an all or none phenomenon. However, its underlying mechanism needs to be clarified.

Ma huang contains ephedrine, pseudoephedrine and phenylethylamine that possess CNS stimulating effects similar to amphetamine (Glennon and Young, 2000). Ephedrine promotes the release of noradrenaline and inhibits the uptake of noradrenaline, resulting in the decrease of food intake and the promotion of satiety (Astrup et al., 1995; Carek and Dickerson, 1999). Furthermore, ephedrine enhances thermogenesis and increases energy expenditure, which intervene weight gain. Ephedrine mediates thermogenic effect by acting on β-adrenergic receptors (Dullo, 1993; Carek and Dickerson, 1999).

The present results showed that Ma huang suppresses the food-deprivation-induced enhancement of the NPY expression in the PVN and ARN of the rat hypothalamus. Based on these results, it is possible that Ma huang is effective in curbing the desire for food by suppressing the NPY expression under food-deprivation conditions.
Acknowledgments

This study was supported by a grant of the Oriental Medicine R&D Project, Ministry of Health and Welfare, Republic of Korea (HMP-02-PJ9-PG3-20600-0003).

References


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