Expressional Changes of Neuropeptide Y and Cholecystokinin in the Arcuate and Paraventricular Nuclei after Capsaicin Administration

In Se Lee1, Young Sam Nam1, Choong Hyun Lee1, Dae Won Chung1, Yeo Sung Yoon1, Jin Sang Kim2, Seong Joon Yi3, Tongkun Pai4 and Heungshik S. Lee1, *

1 Department of Anatomy and Cell Biology, College of Veterinary Medicine and School of Agricultural Biotechnology, Seoul National University, Seoul 151-742, Korea
2 Department of Physical Therapy, College of Rehabilitation, Daegu University, Daegu 705-714, Korea
3 Department of Anatomy, College of Veterinary Medicine, Kyungpook National University, Daegu 702-701, Korea
4 Department of Food and Nutrition, Anyang University, Anyang 430-714, Korea

(Received September 5, 2003)

Summary Despite its toxicity, a great deal of attention has been paid to the anorexic effect of capsaicin in the treatment of obesity-related neurotransmitters/neuromodulators. To determine if capsaicin has any effects on the orexigenic or anorexigenic peptides, the neuropeptide Y (NPY) and cholecystokinin (CCK)-immunoreactivities were demonstrated in the rat hypothalamus by immunohistochemistry after capsaicin administration. There was a significantly lower concentration of NPY immunopositive cells in the arcuate and paraventricular nuclei of the capsaicin treated rats. In contrast, the CCK expressions level was higher in the paraventricular nucleus of the capsaicin treated rats than in the control rats. These results suggest that capsaicin influence neuropeptides such as orexigenic NPY and anorexigenic CCK related to control food intake.

Key Words neuropeptide Y, cholecystokinin, capsaicin, paraventricular nucleus, arcuate nucleus

Capsaicin, the major pungent agent present in hot red pepper, has long been used as an important ingredient in most spicy foods (1). Since its initial identification in 1919, numerous pharmacological effects have been reported and intensively studied (1-3). It is known that adding capsaicin to high-fat meals decreases the appetite while also lowering the perineal adipose tissue weight in rats in a dose-dependent manner (3, 4). Moreover, in the aspect of the appetite and energy intake, capsaicin decreases the subsequent protein and fat intakes as well as the appetite in humans (3).

Many studies have been performed on the specific mediators, receptors and neuronal circuits that regulate the food intake and body weight related to obesity. Peptidergic feeding-regulating neurons have been found in both the hypothalamus and the brainstem, where they act as input stations for hormonal and gastrointestinal information (5).

The hypothalamus has been reported to regulate many aspects of energy homeostasis, adjusting both food intake and energy expenditure in response to various nutritional and other signals (6). In many hypothalamic nuclei, both the arcuate nucleus (ARC) and the paraventricular nucleus (PVN) are related with the important centers that regulate food intake (5, 7). The ARC, which is located in the mediobasal hypothalamus, has been implicated with the control of the feeding behavior by a number of different approaches. Broberger and Hökfelt (5) reported that the ARC could function as a metabolic sensor, mediating the endocrine information that controls the supply and demand of energy in the body. The PVN regulates several responses during the periods of altering energy availability because it possesses the anatomically proper projections to the autonomic and endocrine control sites involved in maintaining homeostasis (8). These nuclei exert their functions through various neurotransmitters such as neuropeptide Y (NPY), cholecystokinin (CCK), agouti-related protein (AgRP) and orexin, by affecting the feeding and energy expenditures (5-7, 9, 10).

Among these neurotransmitters, NPY, a peptide consisting of 36-amino acid residues and a member of the pancreatic polypeptide family, is distributed abundantly throughout the mammalian brain (11). NPY is synthesized throughout the brain, but particularly abundant in the hypothalamus (12). Increases in both biosynthesis and the release of NPY along the distinct neuronal circuits constitute a specific hypothalamic response to the stimulation of food intake (13). Therefore, NPY is believed to be a key neurotransmitter in the regulation of food intake and energy homeostasis (14, 15).

CCK, a 33-amino acid polypeptide, was originally isolated from the duodenum and is widely distributed in the CNS of many species (9, 16). CCK is present in significant amounts in the brain, and acts as either a neu-
Capsaicin on Expressional Changes of Neuropeptides

Fig. 1. NPY-immunoreactive neurons in the arcuate nucleus of the control (A, C) and capsaicin treated (B, D) groups after colchicine treatment. The number of NPY-immunoreactive neurons decreased remarkably in the capsaicin treated group as compared to the control group. Bar=A, B (100 μm), C, D (50 μm).

rotransmitter or neuromodulator (9, 16). In contrast to NPY, CCK can inhibit food intake and act as a central satiety factor in most mammalian species including the rhesus monkey and humans (16).

Therefore, it was fully anticipated that the anorexic or orexic functions of the hypothalamic neuropeptides would be altered by capsaicin. In this study, the expressional changes of NPY and CCK after capsaicin treatment in the ARC and PVN of the rat hypothalamus were examined in order to identify if capsaicin has any anorexic effects on the obesity-related neuropeptides.

MATERIALS AND METHODS

Male Sprague-Dawley rats, 9–10 wk of age, weighing 300±20 g, were obtained from Daehan Biolink (Ram-sung, South Korea). They were housed individually in a light- and temperature-controlled room (12:12-h light-dark cycle, 24±1°C, respectively). The animals were provided with a diet and water ad libitum, and housed for at least 2 wk prior to the experiments. All experiments were carried out within the normal range of the housing temperature. The animal care and all the experimental methods used in this study were approved by the Animal Care and Use Committee at SNU and conformed to the NIH guidelines.

Capsaicin (8-methyl-N-vanillyl-6-nonenamide, Sigma, St. Louis, USA) was given as a vehicle solution consisting of Tween 80 (polyoxyethylenesorbitan monooleate; Sigma), absolute ethanol and saline (10:10:80, v/v). The experimental animals (n=20) were intraperitoneally injected with a dose of 5 mg/kg capsaicin, respectively, and the control animals (n=20) were injected with only an equal volume of the vehicle. Half of each group was treated with a stereotaxical intracerebroventricular infusion of colchicine (50 μg/B.W. 100 g) over a period of 10 min under anesthesia with mixture of ketamine and xylazine (50 mg/kg, 10 mg/kg, respectively) 6 h after the capsaicin injection. Forty-eight hours after the colchicine treatment, the animals were reanesthesized with a mixture of ketamine and xylazine (50 mg/kg, 10 mg/kg, respectively), and perfused via the ascending aorta with 0.1 M phosphate-buffered saline (PBS) followed by 4% paraformaldehyde in 0.1 M PBS (pH 7.4). The brains were removed, and post-fixed in the same fixative for 4 h. The brain tissues were cryo-protected by an infiltration with 30% sucrose over night. Subsequently, the tissues were frozen and sectioned with a cryostat at 30 μm, and consecutive sections were collected in six-well plates containing 0.1 M PBS.

For the immunohistochemical stain, the free-floating sections were incubated with 10% normal goat serum for 15 min at room temperature. These sections were then incubated in rabbit anti-NPY (diluted 1:2,000, Chemicon, USA) or rabbit anti-CCK-8 (diluted 1:1,000, Serotec, UK) antisera overnight at room temperature. The antisera were diluted with 0.1 M PBS containing 0.3% Triton X-100 and 2% normal goat serum. After washing five times for 7 min, the sections were incubated sequentially in goat anti-rabbit IgG (Vector, USA)
and streptavidin (Vector), diluted 1:200 in the same solution as the primary antiserum. The tissues were then washed five times with 0.1 M PBS for 7 min after each incubation step. The sections were then visualized with DAB (3,3'-diaminobenzidine tetrahydrochloride, Sigma) in 0.1 M Tris buffer (pH 7.4) containing 0.003% hydrogen peroxide and mounted on gelatin-coated slides. The immunoreactivities for NPY and CCK in the ARC and PVN were observed using an Axioplan microscope (Carl Zeiss, Germany).

Images of NPY and CCK immunoreactivity in the hypothalamus of each animal were captured using an Applescanner (Apple, USA). The number of NPY and CCK immunoreactive neurons was counted using Optimas 6.5 software (MediaCybernetics, USA). Student’s t-test was performed for statistical significance of immunopositive cells.

RESULTS

NPY-like immunoreactivity

NPY-like immunoreactivity (NPY-LI) was observed in the cell bodies and fibers of the ARC both in colchicine (Fig. 1) and non-colchicine treated rats, but the density of NPY-LI was remarkably lower in the non-colchicine group (data not shown). However, NPY-LI of the PVN was demonstrated only in the fibers of the non-colchicine rats (Fig. 2).

The numbers of NPY immunoreactive neurons in the ARC decreased markedly from 135.8 ± 10.7 in the control group to 61.7 ± 8.8 in the capsaicin treated group.

Fig. 2. Distribution of NPY-immunoreactive fibers in the paraventricular nucleus of the control (A) and capsaicin treated (B) groups. The number of NPY fibers was lower after capsaicin treatment. Bar=100 μm.

Fig. 3. Expossional changes of CCK-immunoreactive neurons in the PVN of the control (A) and capsaicin (B) treated groups after colchicine treatment. CCK-immunoreactivity was increased as a result of capsaicin treatment. Bar=A, B (100 μm), C, D (50 μm).
NPY-LI in the PVN fibers was also lower in the capsaicin treated group than in the control group.

**CKK-like immunoreactivity**

CKK-like immunoreactivity (CKK-LI) was found only in the neurons of the PVN in the colchicine treated rats (Fig. 3). In the ARC, no CKK-LI was observed in either the colchicine or the non-colchicine treated groups (data not shown).

The number of CKK immunoreactive neurons in the PVN increased considerably from 75.70±11.9 in the control group to 135.6±16.9 in the capsaicin treated group. The CKK-LI density in the PVN was also higher both in the cell bodies and fibers of the capsaicin treated animals.

**DISCUSSION**

Many studies have reported that the food-intake conditions such as feeding and fasting influence the NPY and CKK content in the CNS. Also, NPY and CKK concentrations in specific brain areas are altered by feeding and fasting, respectively (13, 17). Moreover, NPY and CKK are known to function as signal mediators, regulating food intake and the body weight balance in the hypothalamus (14–16).

NPY is believed to be a powerful inducer of feeding and obesity, stimulating feeding on its own and releasing other orexigenic agents, such as AgRP and orexin (6). Exogenous NPY, particularly when injected intracerebrally, stimulates a strong feeding behavior in experimental animals, considerably increasing food intake (18). In the hypothalamus, NPY is synthesized primarily in the ARC, where the NPY neurons project their axons to several other hypothalamic nuclei including the PVN (6, 12).

In this study, the NPY-LI immunoreactivity (NPY-LI) was altered in both ARC and PVN after capsaicin injections. NPY-LI was observed in many neurons of the ARC in both the colchicine and non-colchicine treated animals. However, the immunointensity appeared weak in the non-colchicine treated animals. The number of NPY-immunoreactive neurons of the ARC decreased markedly after capsaicin treatment. These results suggest that capsaicin affects NPY expression in the ARC neurons. In contrast to the ARC, NPY-LI of the PVN was observed only in the fibers of the non-colchicine treated rats.

The physiological role of the ARC neurons is to sense and respond to negative energy balance states, and for the NPY neurons of the ARC, by releasing NPY into the PVN, to protect against starvation and contribute to hyperphagia and obesity (6). Accordingly, the diminishment of NPY expression by capsaicin administration, as confirmed in this study, suggests that capsaicin may relate to food intake by affecting the NPY neurons in the ARC.

CKK is a satiety signal (19, 20), and endogenous central CKK is believed to act as a central satiety factor (16). It has been reported that exogenously administered CKK inhibits food intake in rats, and that the behavioral changes caused by CKK are consistent with the production of satiety (18). Moreover, alteration of the feeding status might change not only the brain CKK levels, but also the CKK receptor concentrations in specific regions of the mouse brain (20). Moran (10) suggested that CKK affects food intake by itself and/or by interactions with other hormones or neuropeptides, and that the regulation of food intake is dependant on alterations of the CKK sensitivity. Ingram et al. (21) reported that CKK and CKK mRNA exist in some hypothalamic nuclei including the PVN. It is also known that the gastric vagal afferents, which are related to the satiety effect of CKK, terminate the cell bodies in the nucleus tractus solitarius, the axons that project to the PVN (12).

In this study, CCK-like immunoreactivity (CCK-LI) was observed only in the PVN of the colchicine treated rats, but not in the ARC in any of the experimental groups. The number of CCK immunoreactive cell bodies and fibers in the PVN was higher in the capsaicin treated rats (approximately 2-fold) than in the control group. The enhanced CCK of the PVN suggests that capsaicin increases in the anorexigenic effect of CCK. Baldwin et al. (16) reported that the PVN is an important target site for mediating the hypophagic actions of CCK. They suggested that PVN lesions abolish the inhibition of the food intake by CCK administration. In this study, it was not confirmed whether or not the CCK of PVN was synthesized by PVN itself. However, it could be postulated that the enhanced CCK-LI of the PVN after capsaicin treatment promote the anorexigenic function of CCK.

The results of this study indicate that the administration of capsaicin decreases the orexigenic NPY expression, but increases the anorexigenic CKK expression in the neurons of the hypothalamic nuclei. The effect of capsaicin described in this study supports that reported in previous studies, which showed that capsaicin is closely related to reduced food intake and adipose tissue weight (3).

In summary, these results give anatomical support for the hypothesis that capsaicin plays an inhibitory role in food intake by changing the NPY and CCK expression pattern in the hypothalamic nuclei.

**Acknowledgments**

This work was supported by grant No. R01–2000–000–00159–0 from the Basic Research Program of the Korea Sciences & Engineering Foundation.

**REFERENCES**


4) Ohnuki K, Haramizu S, Oki K, Watanabe T, Yuzawa S.


