원 저

Effects of Electroacupuncture on Food Intake and mRNA Expressions of the Hypothalamic Cholecystokinin in Rats

Woo Hyun-su, Kim Yong-snk and Kim Chang-hwan

Department of Acupuncture & Moxibustion, College of Oriental Medicine, Kyung-Hee University

국문초록

전침이 흰쥐에서 먹이섭취와 시상하부의 콜레시스토키닌 mRNA 발현에 미치는 영향

우현수 · 김용석 · 김창환

경희대학교 한의과대학 침구학교실

목적 : CCK는 현재까지 가장 많이 연구된 식후의 포만신호 전달물질로, 음식섭취를 감소하고, 캡사이신 반응성의 미주신경에 의해 위장운동과 위내의 공복감을 억제시킨다. 전침의 진통효과 발현기전에 영향을 미치는 항아편양 단백물질로서, 내인성 CCK와 그 수용체(CCK-A와 CCK-B)의 역할은 기존의 연구에서 이미 보고되어 왔다. 이에 착안하여, 본 연구에서는 포만감의 측면에서 전침자극이 내인성 CCK의 발현에 어떠한 영향을 미치는가를 살펴보고자 한다.

방법 : 48시간 절식 쥐 모델을 이용하여, 전침자극 후 30분과 60분동안, 먹이 섭취량 변화를 측정하고, 먹이섭취량에 영향을 주는 신경전달경로에서 CCK가 관여하는지를 확인하기 위해 미주신경절제술을 시행한 쥐와 비교하였다. 한편 48시간 절식 쥐 모델을 대상으로 하여 침자극후 시상하부의 CCK mRNA 발현변화를 관찰하였다.

결과 : 전침군에서 30분과 60분 뒤의 먹이 섭취가 대조군에 비해 현저히 낮게 관찰되었는데, 포만감에 관련된 침의 이와 같은 효과는 CCK 수용체에 길항작용이 있는 lorglumide와 미주신경절제술에 의해 차단됨을 알 수 있었다. 시상하부의 CCK mRNA의 발현도는 대조군에 비하여 전침군에서 증가하는 경향이 관찰되었으나, 통계학적인 유의성은 확인할 수 없었다.

· 접수 : 2007년 3월 16일 · 수정 : 2007년 3월 23일 · 채택 : 2007년 3월 23일
· 교신저자 : 김창환, 서울특별시 동대문구 회기동 1번지 경희의료원 한방병원 침구과
Tel. 02-958-9192 E-mail : Kcuacu@khmc.or.kr

결론 : 위의 결과에서, 전침은 포만감에 영향을 미치는 내인성 CCK 메카니즘을 활성화시키는 것을 알 수 있었다.

핵심 단어 : electroacupuncture (EA), cholecystokinin (CCK), satiety, food intake, hypothalamus

I. Introduction

Appetite and satiety are important factors in the condition of feeling hunger and eating food. Although an exception like ghrelin has recently been discovered, most gastrointestinal(GI)-generated signals that influence meal size cause less food to be eaten, consistent with the term satiety signals[1,2]. Satiety signals are released from the specialized enteroendocrine cells that are interspersed among the gastric and intestinal cells lining the lumen of the GI tract[3]. They are transmitted by a subset of meal related metabolites, monoamines, and peptides either to the nucleus of the solitary tract(NTS) via vagal afferent pathway and/or to the hypothalamus via bloodstream[4].

Cholecystokinin(CCK) is a peptide that is found throughout the brain and in neurons and endocrine cells of the GI tract. It plays a variety of roles in coordinating gastrointestinal activity and has been demonstrated to be an important mediator for the control of meal size[5]. The remarkable function of CCK as a satiety signal is to reduce food intake and inhibit gastric motility and emptying which are mediated through capsaicin-sensitive vagal sensory neurons[6,7]. Previous studies have been showed CCK transmits its satiety signal to the brain via the afferent limb of the vagus nerve[8,9], so subdiaphragmatic vagotomy or midbrain transections to cut ascending afferent fibers of the NTS block CCK-induced feeding reduction[10,11].

Acupuncture has long been used in Eastern Asian countries for the treatment method of various diseases including pains and paralyses with few side effects and it is recently considered a new alternative method of medicine in Western countries[12,13]. Electroacupuncture(EA) is a modified technique of acupuncture using electrical stimulation[14] and it is used to treat a variety of diseases including body weight control of normal or obese subjects[15,16]. The potent analgesic effects of EA in various animal species were mediated by release of endogenous opioid peptides and/or by the descending inhibitory control in the central nervous system(CNS)[17,18]. Previous studies showed that there were certain relations between endogenous opioid mechanism of EA analgesia and endogenous anti-opioid peptide CCK and its receptors(CCK-A and -B)[19-21]. Also, it was reported that the EA-induced body weight reduction was mainly due to a decrease in food intake rather than an increase in energy expenditure[22]. So we were interested in the relationship between reduction of food intake induced by EA and endogenous CCK expressions in view of a satiety signal.

To investigate peripheral endogenous CCK expressions, we examined the amounts of food intake after EA stimulation in 48-hr fasted rats and compared the changes of food intake when the lorglumide were injected in the same models. To confirm that vagus nerve mediate the satiety signal of CCK, we compared the amounts of food intake between vagotomized rats and sham operated rats. To investigate central endogenous CCK expressions, we observed the mRNA expressions of the hypothalamic CCK in 48-hr fasted rats.

Ⅱ. Materials and Methods

1. Experiment animals

Male Sprague-Dawley rats(Sam : TacN(SD) BR, 6~8 weeks), weighing 250±25g were housed in plastic cages(three or four rats in a cage), given free access to water, and maintained on a 12hr light-dark cycle(08:00-20:00 hr light; 20:00-08:00 hr dark) in proper temperature(25±2℃). All procedures involving animals were conducted in accordance with NIH guidelines. All rats used in experiments were sufficiently habituated to handling, holder restraint, and acupuncture insertion. They were handled daily from 5days before the beginning of the experiment and were adapted to acupuncture insertion under holder restraint every other day during the above handling period.

2. Experiment groups

To examine the amounts of food intake after EA stimulation and to observe the mRNA expressions of the hypothalamic CCK, we randomly divided rats into four groups as follows (n=9~11rats/group); control group(fasting), holder group(fasting+holder restraint), EA group (fasting+EA stimulation at ST_{36}) and non-acupuncture point group(NA group, fasting+EA stimulation at a non-acupuncture point).

To confirm that vagus nerve mediate the satiety signal of CCK, we randomly divided rats into two groups as follows (n=6rats/group); vagotomy group(subdiaphragmatic vagotomy+fasting+EA stimulation at ST_{36}), sham surgery group (Sham surgery+fasting+EA stimulation at ST_{36}).

3. Holder stress and electroacupuncture

Rats of all groups except control group were lightly immobilized using a plastic holder restrainer (5.3×15, 5.6×17, 6.0×24cm in diameter×length, Tae Yang Co. Korea). The rats in holder group were restrained for 30-min without acupuncture insertion. For the treatment of acupuncture in EA and NA groups, we used stainless steel acupuncture needles(0.25mm in diameter and 3cm in length, Dong Bang Co. Korea) and electrical stimulators (PG-306, Suzuboku Co. Japan). The depth of needle insertion was about 5 mm for each subject.

In EA group, we selected ST36 and the point of 5 mm apart below ST36 as acupuncture points. Previous studies showed that EA stimulation at ST36 elevated the secretion of somatostatin and CCK[23] and reduced body weight and food intake11). ST36 is located on a borderline between proximal 1/5 and 2/5 part among the division into 5 equal parts from ST35 up to anterior surface of ankle joint, level with the lateral area to anterior of tibia in rat[24]. Anode lead connected to ST36, cathode lead connected to the point of 5 mm part from ST36, then train-pulses with high frequency (100Hz, 0.3ms pulse width, 0.2-0.3mA) were applied for 30-min.

In NA group, we selected two non-acupuncture points of the tail. One was 2cm apart from proximal part of tail and the other was 1cm apart from the first selected point. Anode lead connected to the upper selected point and cathode lead connected to the lower selected point. The electric current was applied with the same method as EA group.

4. Vagotomy and sham surgery

Bilateral subdiaphragmatic vagotomy was performed as previously described[10,11]. In brief, a midline incision was made to provide wide exposure of the upper abdominal organs and gentle traction was placed on the stomach to pull a portion of the thoracic esophagus into abdomen. After the bilateral subdiaphragmatic trunks of vagus nerve (dorsal and ventral branches) were exposed along the right and left sides of the esophagus with an operating microscope, the trunks were split and cut. In sham surgery, the bilateral trunks of vagus nerve were only exposed

and split. To verify the success of vagotomy, it required at least 2weeks because the rats are supposed to fully recover from the surgery in 2 weeks. To confirm whether the vagotomy surgery had been successful, we administered ghrelin(1.5 mmol/rat) intravenously to the vagotomized and sham operated rats at the light phase and measured food intakes at 2-hr later. Ghrelin, a peptide hormone made in the stomach, is supposed to increase appetite and the signal is transmitted via vagus nerve[25], therefore blockade of ghrelin-induced feeding increase in the vagotomized rats indicated that the vagotomy surgery was successful[4]. In the measurement of food intake at 2-hr later after grelin injection, the amounts of food intake of vagotomized rats were 0.60±0.11 g whereas those of sham operated rats were 2.33±0.12g. There were significant difference between vagotomy group and sham surgery group ($P < 0.001$), so we considered the vagotomy surgery was successful.

5. Measurement of food intake

During the fasting period, tap water was given sufficiently to prevent dehydration. The day up to settled time(48-hr) from starting fasting, EA stimulation was performed at 5:00/pm. Before checking the amount of food intake, the cubed diet(chow) given to the each group was prepared equivalently. At 30-min after EA stimulation, we started feeding chow to the rats of control, holder, EA and NA groups and measured the amounts of food intake at 30-min, 60-min later. In the next experiment, to verify the relationship between EA stimulation and CCK expressions, Lorglumide(a CCK-A receptor antagonist , dissolved in 0.9% normal saline) was administered intra-peritoneally (i.p) with 10mg/kg dose at 15-min before EA stimulation in the EA group. And we measured the changes of food intake as the same method. In sequent experiment, to confirm that vagus nerve mediate the satiety signal of CCK, we measured the amounts of food intake at 30-min, 60-min later as the same method in vagotomy group and sham surgery group.

6. Assay of mRNA expressions of hypothalamic CCK

Specimens for RNA analysis were obtained from the hypothalamus, and immediately frozen in liquid nitrogen, and stored at -80℃. Frozen specimens were homogenized with a Polytron homogenizer and total RNA was extracted by the acid guanidium isothio cyanate-phenol-chloroform (AGPC) method. First-strand cDNA was synthesized from total RNA with M-MuLV Reverse Transcriptase (Fermentas, Canada). Approximately 1mg of total RNA was used as the template to synthesize cDNA with 10 U of reverse transcriptase in reaction mixtures containing 1.25 pM of oligo (dt) primer, 1 mM dNTP and 10 U of ribonuclease inhibitor. Reverse transcription was performed at 30℃ for 10 min and then at 42℃ for 20 min, at 99℃ for 5min, and at 4℃ for 5min. Quantitative PCR was performed on a GeneAmp 9700 Sequence detection system(Applied Biosystems, Warrington, UK). Amplification was carried out in a total volume of 20ml containing 10mM of each specific primer(Table 1), 2units of taq DNA polymerase and 2ml of diluted cDNA. Dilution of cDNA was carried out depending on the initial concentration of each cDNA sample to render the concentration of each cDNA commensurate to one another. The PCR reactions were cycled 40times through denaturation (95℃, 15sec) and annealing (60℃, 1 min), and extension (72℃, 30sec). The images of all samples were captured by SL-20 High Performance DNA Image Visualizer(SLB mylmager, SeouLin Bioscience Co.Ltd, Korea) and the density of the band was measured by using image acquisition and analysis Software(LabWorks, UVP, Upland, CA). In addition, mRNA levels of the androgen-regulated house keeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were measured.

Table 1. Sequences of primers used for PCR

Gene	Primer sequence
GAPDH	Sense: 5′-GGC ATG GAC TGT GGT CAT GA-3′ Antisense: 5′-TTC ACC ACC ATG GAG AAG GC-3′
CCK	Sense: 5′-AGC CGG TAG TCC CTG TAG AA-3′ Antisense: 5′-GTC CCG GTC ACT TAT CCT GT-3′

7. Statistical analysis

Data were presented as mean±SEM(standard error mean). Statistical analysis was performed with GraghPad Prism® windows 3.02(GraghPad Software, Inc., USA). In the experiment to examine the amounts of food intake after EA stimulation, and in the experiment to observe the mRNA expressions of the hypothalamic CCK, we used the Kruskal-Wallis test followed by Dunn's multiple comparison test for statistical comparisons. In the experiment to confirm that vagus nerve mediate the satiety signal of CCK, we used the unpaired t-test for statistical comparisons. $P < 0.05$ was considered significant.

Ⅲ. Results

1. Food intake measurement

In the experiment to examine the amounts of food intake after EA stimulation in 48-hr fasted rats, the amounts of food intake at 30-min later in EA group were significantly lower than the amounts of food intake in control group, and the amounts of food intake at 60-min later in EA group were also significantly lower than the amounts of food intake in control group(Fig. 1).

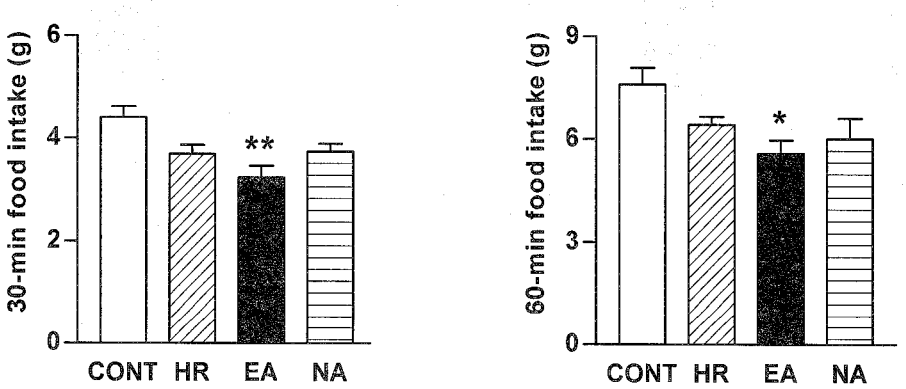

Fig. 1. The effects of EA stimulation on food intake in 48-hr fasted rats
Data was presented as mean±SEM. Statistical comparisons between four respective experimental groups (n=9~11 rats/group) were performed with Kruskal-Wallis test followed by Dunn's multiple comparison test. *$P < 0.05$, **$P < 0.001$ compared with control group. CONT; control group, HR; holder group, EA; electroacupuncture group, NA; non-acupuncture point group.

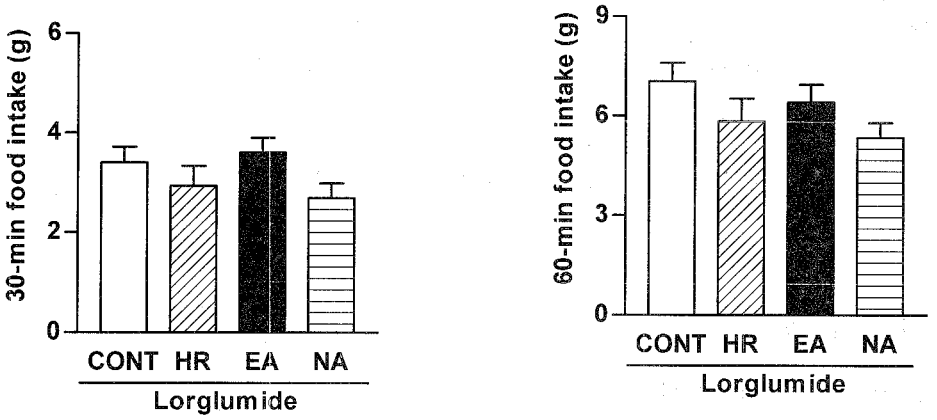

Fig. 2. The effects of lorglumide(10mg/kg, i.p.) pretreatment on EA-induced reduction of food intake in 48-hr fasted rats

Data was presented as mean±SEM. Statistical comparisons between four respective experimental groups(n=9~11 rats/group) were performed with Kruskal-Wallis test followed by Dunn's multiple comparison test. CONT; control group, HR; holder group, EA; electroacupuncture group, NA; non- acupuncture point group.

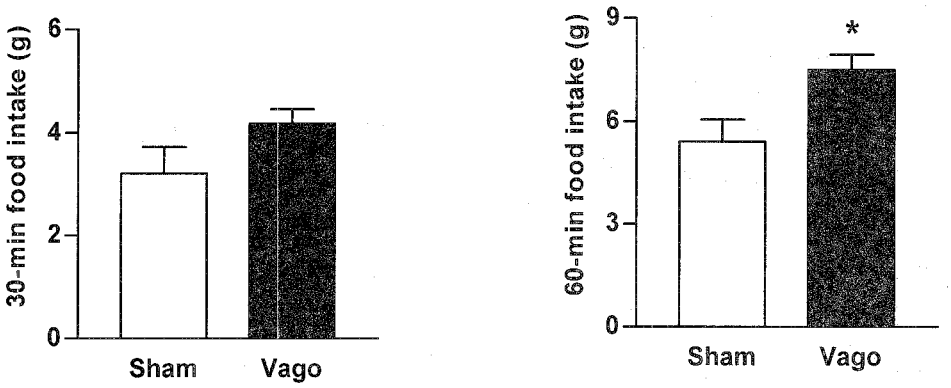

Fig. 3. The effects of vagotomy on the EA-induced reduction of food intake in 48-hr fasted rats

Data was presented as mean±SEM. Statistical comparisons between two experimental groups (n=6 rats/group) were performed with the unpaired t-test. *P < 0.05 compared with sham group. Sham; sham surgery group, Vago; vagotomy group.

These effects were completely blocked by lorglumide(CCK-A receptor antagonist) pretreatment (Fig. 2). Although the amounts of food intake at 30-min later in holder and NA groups were decreased, neither group represented a statistically significant reduction versus control group.

In the experiment to confirm that vagus nerve mediate the satiety signal of CCK, the amounts of food intake at 30-min later were not significantly different between vagotomy group and sham surgery group, but the amounts of food intake at 60-min later in vagotomy group were significantly increased versus sham surgery group(Fig. 3).

2. mRNA expressions of the hypothalamic CCK

CCK mRNA levels were normalized versus Glyceraldehyde-3-phosphate dehydrogenase(GAPDH) mRNA. The semi-quantitative PCR analysis showed

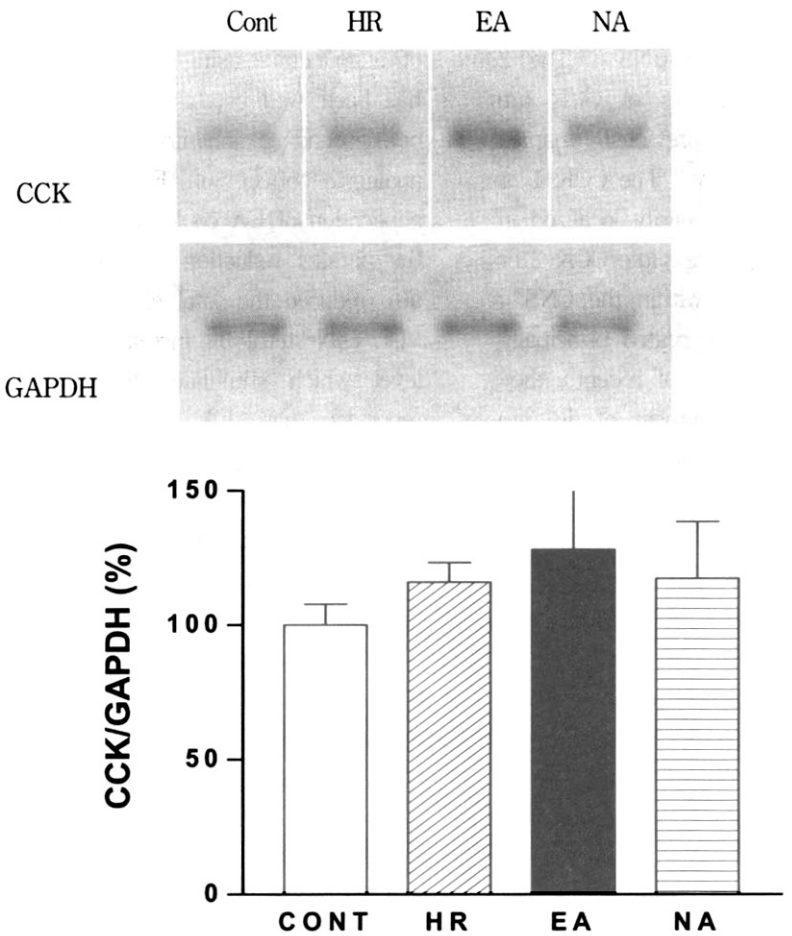

Fig. 4. The representative images of mRNA expressions of hypothalamic CCK and GAPDH(upper panel), and mRNA levels of hypothalamic CCK normalized GAPDH mRNA in 48-hr fasted rats (lower panel)
Data are presented as mean±SEM. Statistical comparisons between four respective experimental groups (n=6 rats/group) were performed with Kruskal-Wallis test followed by Dunn's multiple comparison test. CONT; control group, HR; holder group, EA; electroacupuncture group, NA; non-acupuncture point group.

that the mRNA expressions of the hypothalamic CCK were increased in EA group, although there were no statistical significant difference between four experimental groups(Fig. 4).

Ⅳ. Discussion

Feeding regulation in view of appetite and satiety is very closely related with endocrine hormones and peptides. For example, if the levels of leptin, CCK, norepinephrine and corticotropin releasing hormone are increased in serum, human will lose weight[26] and if the levels of ghrelin, Neuropeptide Y and cortisol are increased, humans will gain weight. Several different types of enteroendocrine cells have been identified and are categorized by the peptide signals that they synthesize and secrete[1].

Among these, CCK is the most studied postprandial satiety signal and is released from small intestinal endocrine cells and participates in the control of a variety of gastrointestinal and behavioral activities[27]. It is secreted primarily in 2 forms. One is derived from the intestinal I cells

within the duodenal and jejunal mucosa[28] and the other is synthesized within the CNS[29]. The 2 receptors that mediate the effects of CCK are termed CCK-1 and CCK-2, previously named CCK-A and CCK-B, respectively[5]. The CCK-1 or A(for alimentary) receptor is primarily localized in the gastrointestinal system, whereas the CCK-2 or B(for brain) receptor is found within the CNS[30]. Although CCK-A receptors are located principally in the peripheral nervous system of rodents, they have also been detected in a number of discrete regions of the brain[31]. Lorglumide (L-364,718) is an antagonist that has a high affinity for CCK-A receptors[32,33].

The satiety signal of CCK plays an essential role in the short-term regulation of feeding[34], and is transmitted to the brain through special receptors located in the vagal capsaicin- sensitive afferent neurons[35]. The vagus nerve is a mixed nerve containing primary afferent fibers which transmit viscero-sensory information from the gastrointestinal tract and cardio-respiratory organs[36]. The cell bodies of these fibers are located in the nodose ganglion(NG), and central branches terminate in the nucleus of the solitary tract[37], where this information is relayed onto second-order sensory neurons projecting to multiple brain sites[38]. Repeatedly, vagal afferent neurons are a major avenue by which food related signals from the gastrointestinal(GI) tract access the brain to control feeding behavior. Although the chemical and colligative properties of ingesta may directly activate vagal afferents, compelling evidence indicates that ingesta trigger endocrine and paracrine secretions from the gastrointestinal mucosa, and that these neuroactive secretions activate or modulate vagal afferent activity[39]. Vagal afferent neurons may respond directly to mechanical, chemical, or inflammatory stimuli, the gut-peptide CCK acts as one of these stimuli and brings about reduction of food intake[40].

Acupuncture has been used as a therapeutic treatment for various kinds of pain including lower back pain, chronic elbow pain, myofascial pain, toothache, etc[41]. EA is a modified technique of acupuncture using electrical stimulation and it has been well established that endogenous opioid or descending inhibitory pathways mediate the analgesic effect of EA in the CNS[42]. The application of EA could reduce the body weight in the parallel reduction with the leptin level[43] and also reduced the total cholesterol, triglyceride and LDL cholesterol by increasing the beta-endorphin level which stimulates lipolysis[44]. Also it was reported that EA reduced starvation-induced stress and suppressed expression of gastric ghrelin and hypothalamic NPY and the EA-induced body weight reduction was mainly due to a decrease in food intake rather than an increase in energy expenditure[22].

As an anti-opioid peptide in the analgesic effect of EA, the role of endogenous CCK and its receptors have also been documented in a previous study[45]. Especially, high frequency EA stimulation promotes the expressions of hypothalamic CCK and of its receptors[21]. So we had an interest in the relationship between the reduction of food intake induced by EA and endogenous CCK expressions in view of satiety.

To investigate peripheral endogenous CCK expressions, we examined the amounts of food intake after EA stimulation in 48-hr fasted rats and compared the changes of food intake when the lorglumide were injected in the same models. To confirm that vagus nerve mediate the satiety signal of CCK, we compared the amounts of food intake between vagotomized rats and sham operated rats. To investigate central endogenous CCK expressions, we observed the mRNA expressions of the hypothalamic CCK in 48-hr fasted rats.

In the results, the amounts of food intake at 30-min and 60-min later in EA group were significantly lower than the amounts of food intake in control group(Fig. 1). These effects of EA stimulation were completely blocked by lorglumide (CCK-A receptor antagonist) pretreatment(Fig. 2). Although the amounts of food intake at 30-min

later in holder and NA groups were decreased, neither group represented a statistically significant reduction versus control group. These findings suggest that EA stimulation increase the peripheral endogenous CCK expressions which transmitted a satiety signal and it cause the reduction of food intake in 48-hr fasted rats. The effects of EA stimulation were completely blocked by lorglumide pretreatment, whereas lorglumide did not restore the slight reduction of food intake which were observed in holder and NA groups. These findings indicate that the peripheral endogenous CCK affect on the EA stimulation-induced food intake reduction in 48-hr fasted rats.

Since vagus nerves mediate peripheral CCK satiety signals to the brain[46,47], we compared the effects of EA on food intake between vagotomized rats and sham operated rats. The amounts of food intake at 30-min later were not significantly different between vagotomy group and sham surgery group, but the amounts of food intake at 60-min later were significantly increased in vagotomy group(Fig. 3). These findings suggest that vagotomy restore the EA stimulation-induced reduction of food intake in 48-fasted rats. Also, it verify that moderate blockade of EA effects by vagotomy may be due to the abolishment of the effects of other hormones such as ghrelin, PYY_{3-36} and glucagon-like peptide-1 on the vagus nerve[4,48].

Prevous study suggested that CCK could act as a neurotransmitter or neuro-modulator to produce satiety signal in the hypothalamus and hindbrain of the CNS[49]. Therefore, we investigated the mRNA expressions of the hypothalamic CCK by EA stimulation with semi-quantitative PCR analysis. Quantitative gene expression data are often normalized to the expression levels of control or so-called "housekeeping" genes. An inherent assumption in the use of housekeeping genes is that expression of the genes remains constant in the cells or tissues under investigation. Although exceptions to this assumption are well documented, housekeeping genes are of value in fully characterized systems. Glyceraldehyde-3-phosphate dehydrogenase(GAPDH) is one of the most commonly used housekeeping genes used in comparisons of gene expression data[50]. In the present study, CCK mRNA levels were normalized versus GAPDH mRNA. The results of semi-quantitative PCR analysis showed that the mRNA expressions of the hypothalamic CCK were increased in EA group, although there were no statistical significant difference between four experimental groups(Fig. 4). These findings suggest that the central endogenous CCK do not affect on the EA stimulation-induced food intake reduction in 48-hr fasted rats.

V. Conclusions

To investigate whether EA stimulation plays some roles in endogenous CCK expressions which related to a satiety signal, we measured the amount of food intake and observed the mRNA expressions of the hypothalamic CCK in 48-hr fasted rats. The amounts of food intake at 30-min and 60-min later in EA group were significantly lower than those in control group, and these effects of EA stimulation were blocked by lorglumide pretreatment and vagotomy. The mRNA expressions of the hypothalamic CCK were increased in EA group, although there were no statistical significant difference.

These results suggest that EA stimulation affect on the endogenous CCK mechanism which mediated a satiety signal and has a tendency to suppress the appetite and food intake.

VI. References

1. Woods SC. Gastrointestinal satiety signals I. An overview of gastrointestinal signals that influence food intake. Am J Physiol

Gastrointest Liver Physiol. 2004 ; 286 : 7-13.
2. Strader AD, Woods SC. Gastrointestinal hormones and food intake. Gastroenterology. 2005 ; 128 : 175-91.
3. Roth KA, Gordon JI. Spatial differentiation of the intestinal epithelium : analysis of enteroendocrine cells containing immunoreactiveserotonin, secretin, and substance P in normal and transgenic mice. Proc Natl Acad Sci. USA. 1990 ; 87 : 6408-12.
4. Koda S, Date Y, Murakami N, Shimbara T, Hanada T, Toshinai K, Niijima A, Furuya M, Inomata N, Osuye K, Nakazato M. The role of the vagal nerve in peripheral PYY3-36-induced feeding reduction in rats. Endocrinology. 2005 ; 146 : 2369-75.
5. Moran TH, Kinzig KP. Gastrointestinal satiety signals II. Cholecystokinin. Am J Physiol. 2004 ; 286 : 183-8.
6. Raybould HE, Tache Y. Cholecystokinin inhibits gastric motility and emptying via a capsaicin-sensitive vagal pathway in rats. Am J Physiol. 1988 ; 255 : 242-6.
7. Sayegh AI, Ritter RC. Vagus nerve participates in CCK-induced Fos expression in hindbrain but not myenteric plexus. Brain Res. 2000 ; 878 : 155-62.
8. Moran TH, Baldessarini AR, Salorio CF, Lowery T, Schwartz GJ. Vagal afferent and efferent contributions to the inhibition of food intake by cholecystokinin. Am J Physiol. 1997 ; 272 : 1245-51.
9. Havel PJ. Peripheral signals conveying metabolic information to the brain : short-term and long-term regulation of food intake and energy homeostasis. Exp Biol Med. 2001 ; 226 : 963-77.
10. Reidelberger RD, Hernandez J, Fritzsch B, Hulce M. Abdominal vagal mediation of the satiety effects of CCK in rats. Am J Physiol Regul Integr Comp Physiol. 2004 ; 286 : 1005-12.
11. Smith GP, Jerome C, Cushin BJ, Eterno R, Simansky KJ. Abdominal vagotomy blocks the satiety effect of cholecystokinin in the rat. Science. 1981 ; 213 : 1036-7.
12. NIH consensus conference. Acupuncture. JAMA. 1998 ; 280 : 1518-24.
13. Kaptchuk TJ. Acupuncture : theory, efficacy, and practice. Ann Intern Med. 2002 ; 136 : 374-83.
14. Ulett GA, Han S, Han JS. Electroacupuncture : mechanisms and clinical application. Biol Psychiatry. 1998 ; 44 : 129-38.
15. Hsu CH, Hwang KC, Chao CL, Chang HH, Chou P. Electroacupuncture in obese women : a randomized, controlled pilot study. J Womens Health(Larchmt). 2005 ; 14 : 434-40.
16. Lee MS, Hwan Kim J, Lim HJ, Shin BC. Effects of abdominal electroacupuncture on parameters related to obesity in obese women : a pilot study. Complement Ther Clin Pract. 2006 ; 12 : 97-100.
17. Han JS. Acupuncture : neuropeptide release produced by electrical stimulation of different frequencies. Trends Neurosci. 2003 ; 26 : 17-22.
18. Takeshige C, Sato T, Mera T, Hisamitsu T, Fang J. Descending pain inhibitory system involved in acupuncture analgesia. Brain Res Bull. 1992 ; 29 : 617-34.
19. Chen XH, Geller EB, Adler MW. CCKB receptors in the periaqueductal grey are involved in electroacupuncture antinociception in the rat cold water tail-flick test. Neuropharmacology. 1998 ; 37 : 751-7.
20. Han JS, Ding XZ, Fan SG. Cholecystokinin octapeptide (CCK-8) : antagonism to electroacupuncture analgesia and a possible role in electroacupuncture tolerance. Pain. 1986 ; 27 : 101-15.
21. Ko ES, Kim SK, Kim JT, Lee G, Han JB, Rho SW, Hong MC, Bae H, Min BI. The difference in mRNA expressions of hypothalamic CCK and CCK-A and -B

receptors between responder and non-responder rats to high frequency electroacupuncture analgesia. Peptides. 2006 ; 27 : 1841-5.
22. Tian N, Wang F, Tian DR, Zou Y, Wang SW, Guan LL, Shi YS, Chang JK, Yang J, Han JS. Electroacupuncture suppresses expression of gastric ghrelin and hypothalamic NPY in chronic food restricted rats. Peptides. 2006 ; 27 : 2313-20.
23. Zhou Y, Sun YH, Shen JM, Han JS. Increased release of immunoreactive CCK-8 by electroacupuncture and enhancement of electroacupuncture analgesia by CCK-B antagonist in rat spinal cord. Neuropeptides. 1993 ; 24 : 139-44.
24. HK Koh. Transpositional acupoints of the rat. The J of Korea Acupuncture and Moxibustion Society. 1999 ; 16(3) : 115-22.
25. Cummings DE, Shannon MH. Roles for ghrelin in the regulation of appetite and body weight. Arch Surg 2003 ; 138 : 389 - 96.
26. Weller A, Smith GP, Gibbs J. Endogenous cholecystokinin reduces feeding in young rats. Science. 1990 ; 247 : 1589-91.
27. Ritter RC, Covasa M, Matson CA. Cholecystokinin : proofs and prospects for involvement in control of food intake and body weight. Neuropeptides. 1999 ; 33 : 387-99.
28. Polak JM, Bloom SR, Rayford PL, Pearse AG, Buchan AM, Thompson JC. Identification of cholecystokinin-secreting cells. Lancet 1975 ; 2 : 1016-8.
29. Beinfeld MC, Meyer DK, Brownstein MJ. Cholecystokinin in the central nervous system. Peptides 1981 ; 2(Suppl 2) : 77-9.
30. Herranz R. Cholecystokinin antagonists : pharmacological and therapeutic potential. Med Res Rev. 2003 ; 23 : 559-605.
31. Hill DR, Campbell NJ, Shaw TM, Woodruff GN. Autoradiographic localization and biochemical characterization of peripheral type CCK receptors in rat CNS using highly selective nonpeptide CCK antagonists. J Neurosci. 1987 ; 7 : 2967-76.
32. Dourish CT, Hawley D, Iversen SD. Enhancement of morphine analgesia and prevention of morphine tolerance in the rat by the cholecystokinin antagonist L-364,718. Eur J Pharmacol. 1988 ; 147 : 469-72.
33. Martins SR, Oliveira RB, Ballejo G. Activation of neural cholecystokinin-1 receptors induces relaxation of the isolated rat duodenum which is reduced by nitric oxide synthase inhibitors. Braz J Med Biol Res. 2006 ; 39 : 271-5.
34. Crawley JN, Beinfeld MC. Rapid development of tolerance to the behavioural actions of cholecystokinin. Nature 1983 ; 302 : 703-6.
35. Moriarty P, Dimaline R, Thompson DG, Dockray GJ. Characterization of cholecystokinin A and cholecystokinin B receptors expressed by vagal afferent neurons. Neuroscience. 1997 ; 79 : 905-13.
36. Paintal AS. Vagal sensory receptors and their reflex effects. Physiol. 1973 ; 53 : 159-227.
37. Schwartz GJ, Moran TH, White WO, Ladenheim EE. Relationships between gastric motility and gastric vagal afferent responses to CCK and GRP in rats differ. Am J Physiol. 1997 ; 272 : 1726-33.
38. Eastwood C, Maubach K, Kirkup AJ, Grundy D. The role of endogenous cholecystokinin in the sensory transduction luminal nutrient signals in the rat jejunum. Neurosci Lett. 1998 ; 254 : 145-8.
39. Ritter RC. Gastrointestinal mechanisms of satiation for food. Physiol Behav. 2004 ; 81 : 249-73.
40. Peters JH, Simasko SM, Ritter RC. Modulation of vagal afferent excitation and reduction of food intake by leptin and cholecystokinin. Physiol Behav. 2006 ; 89 : 477-85.
41. Pomeranz B, Cheng R, Law P. Acupuncture reduces electrophysiological and behavioral responses to noxious stimuli : pituitary is

implicated. Exp Neurol. 1977 ; 54 : 172- 8.
42. Ulett GA, Han S, Han JS. Electroacupuncture : mechanisms and clinical application. Biol Psychiatry. 1998 ; 44 : 129-38.
43. Cabioglu MT, Ergene N. Changes in serum leptin and beta endorphin levels with weight loss by electroacupuncture and diet restriction in obesity treatment. Am J Chin Med. 2006 ; 34 : 1-11.
44. Cabioglu MT, Ergene N. Electroacupuncture therapy for weight loss reduces serum total cholesterol, triglycerides and LDL cholesterol levels in obese women. Am J Chin Med. 2005 ; 33 : 525-33.
45. Ding XZ, Fan SG, Zhou JP, Han JS. Reversal of tolerance to morphine but no potentiation of morphine-induced analgesia by antiserum against cholecystokinin octapeptide. Neuropharmacology. 1986 ; 25 : 1155-60.
46. Schwartz MW, Woods SC, Porte D, Seeley RJ, Baskin DG. Central nervous system control of food intake. Nature. 2000 ; 404 : 661-71.
47. Simasko SM, Ritter RC. Cholecystokinin activates both A- and C-type vagal afferent neurons. Am J Physiol Gastrointest Liver Physiol. 2003 ; 285 : 1204-13.
48. Williams DL, Grill HJ, Cummings DE, Kaplan JM. 2003 Vagotomy dissociates short- and long-term controls of circulating ghrelin. Endocrinology. 2003 ; 144 : 5184-7.
49. Blevins JE, Stanley BG, Reidelberger RD. Brain regions where cholecystokinin suppresses feeding in rats. Brain Res. 2000 ; 860 : 1-10.
50. Barber RD, Harmer DW, Coleman RA, Clark BJ. GAPDH as a housekeeping gene : analysis of GAPDH mRNA expression in a panel of 72 human tissues. Physiol Genomics. 2005 ; 21 : 389-95.