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Electroacupuncture suppresses expression of gastric ghrelin and hypothalamic NPY in chronic food restricted rats

Nan Tian [a,b,1], Fei Wang [a,1], De-Run Tian [c,*], Yuan Zou [b], Shi-Wei Wang [b], Li-Li Guan [b], Yu-Shun Shi [a], Jaw-Kang Chang [d], Jun Yang [d], Ji-Sheng Han [a]

[a] Neuroscience Research Institute, Peking University, Beijing 100083, PR China
[b] Department of Physiology, Dalian Medical University, Dalian 116027, PR China
[c] Department of Anatomy, Tianjin Medical University, Tianjin 300070, PR China
[d] Phoenix Pharmaceuticals Inc., Belmont, CA 94002, USA

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ABSTRACT

Electroacupuncture (EA) has been reported to reduce body weight in overweight subjects in clinical practice, as well as in rats and mice with diet-induced obesity. In the present study, this effect of EA was tested in lean rats subjected to long-term food restriction (FR, food was offered only 1 h/day). Two hertz EA administered once every other day produced a further reduction in body weight in FR rats. Exploration of the mechanism involved revealed significant downregulation of the orexigenic peptides: ghrelin in the stomach, and neuropeptide Y (NPY) but not Agouti-related peptide (AgRP) in the hypothalamus, which was in line with the reduction in food intake in rats receiving EA stimulation as compared with those receiving restraint only. Uncoupling protein 3 (UCP3), involved in accelerating energy expenditure, was not significantly altered. These results suggest that the EA-induced body weight reduction was due mainly to a decrease in food intake rather than an increase in energy expenditure. A reduction in the orexigenic peptides ghrelin and NPY may be involved in the underlying mechanism.

1. Introduction

Acupuncture has a long history in the treatment of many diseases [16]. In recent years, accumulated clinical studies have shown that acupuncture was effective in decreasing the body weight of obese subjects [18,32]. Unlike the mechanisms of EA-induced analgesic effects, which have been relatively thoroughly studied [9], the mechanisms underlying acupuncture-induced body weight change have yet to be explored.

Both central and peripheral mechanisms are involved in energy balance and body weight changes. Ghrelin is a brain-gut peptide with growth hormone-releasing and appetite-inducing activities [17,28]. The amount of ghrelin present in the stomach and in plasma increases before meals and declines afterward, suggesting an important role in meal initiation [1,6]. Mediated by neuropeptide Y (NPY) and Agouti-related peptide (AgRP) in the hypothalamus, ghrelin was predicted to be one of the most potent peripheral signals for controlling energy balance [4,33]. Several lines of evidence have suggested that uncoupling protein 3 (UCP3) is positively associated with energy expenditure [10,26,27]. Mice over-expressing UCP3 are hyperphagic but lean [5]. In addition, UCP3 expression is upregulated in skeletal muscles during both fasting and high-fat feeding [2,7].

* *Corresponding author*. Tel.: +86 22 23542604; fax: +86 22 2354 2735.
E-mail address: tianderun@hotmail.com (D.-R. Tian).
[1] These authors contributed equally to this paper.
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Our previous study showed that the increase in body weight and food intake observed in diet-induced obesity (DIO) rats was effectively reversed by peripheral electrical stimulation. This was associated with the upregulation of anorexigenic peptides such as cocaine- and amphetamine-related transcript (CART) and α-melanocyte-stimulating hormone (α-MSH) [30]. In the current study, we reveal that EA produced a modulation of body weight in long-term food restriction (FR) rats. Furthermore, we studied the possible mechanisms by observing the changes of the orexigenic peptides—ghrelin in the stomach, and NPY and AgRP in the hypothalamus, as well as the level of UCP3 in the gastrocnemius muscle.

2. Materials and methods

2.1. Long-term food restriction rat model

Forty male Sprague–Dawley (SD) rats (initial body weight 240–250 g) were obtained from Vital Company (Beijing). Animals were maintained in the animal room for 1 week before the starting of the experiment. They were housed in a facility with controlled temperature (22 ± 2 °C) and maintained in 12-h light/12-h dark cycles (light on from 07:00 to 19:00). The ad lib-fed group (n = 10) was provided with full access to regular rat chow. The food restriction group (n = 30) were deprived of food for 23 h and re-fed for 1 h (19:00–20:00) for 18 consecutive days. The FR rats were then randomly and evenly divided into three groups: (1) the 2 Hz EA group, treated with 2 Hz EA half an hour before re-feeding every other day (18:30–19:00); (2) the restraint group, restrained in a special restrainer accompanying the EA group; and (3) the control group, which received no EA stimulation nor underwent any procedures performed on the other two groups. Body weight was recorded at 18:00 and food intake recorded at 20:00 every day.

2.2. EA equipment and parameters

Rats of the restrained group and the 2 Hz EA group were restrained in 30 cm long cylindrical Plexiglas restrainers, and partially immobilized therein during the EA treatment. Holes in the anterior end of the restrainer provided ventilation, while the two rear openings allowed extension of the hind legs of the rat for the placement of acupuncture needles.

A stainless steel acupuncture needle (0.3 mm diameter) was bent into an 'L' shape. The proximal end was soldered to a wire that was connected to one of the output channels of an electric stimulator, ''HANS'' (Han's Acupoint Nerve Stimulator; Neuroscience Research Institute, Peking University, China). For rats of 2 Hz EA group, the distal ends of two needles were inserted into the acupoints on each hind leg: (1) Zusanli (ST36, near the knee joint, 4 mm lateral to the anterior tubercle of the tibia) and (2) Sanyinjiao (SP6, near the ankle joint, at the level of the superior border of the medial malleolus between the posterior border of the tibia and the anterior border of the Achilles tendon). Needles were inserted to a depth of 5 mm at ST36, and just penetrating at SP6. After insertion, the needles were fixed in situ with adhesive tape [30].

The EA parameters were set as follows: constant current square wave output (pulse width 0.6 ms); frequency set at 2 Hz; intensities increasing stepwise from 0.5 to 1.0 and then 1.5 mA, each lasting for 10 min, totaling 30 min.

2.3. Histology

At the end of the experiment, half of the animals in every group were sacrificed by decapitation. Blood samples were collected and plasma was harvested by 15 min centrifugation (3000 rpm) in plasma separator tubes. The hypothalamus was removed by making an incision medial to piriform lobes, caudal to the optic chiasma and anterior to the cerebral crus to a depth of 2.5 mm. The gastrocnemius muscle was dissected and removed quickly. Tissues were frozen in liquid nitrogen and stored at −80 °C until analysis. The peri-renal fat pads were dissected and weighed. The other animals were anaesthetized with sodium pentobarbital (50 mg/kg, i.p.) and perfused transcardially with 150 ml isotonic saline followed by 250 ml 4% paraformaldehyde. Stomachs and brains were removed and post-fixed in the same fixative overnight. The stomachs were embedded in paraffin and 5 μm thick sections were made. Floating brain sections of hypothalamus at 30 μm thickness were prepared.

2.4. Measurement of plasma triglycerides and glucose

Plasma triglycerides and glucose were analyzed in Biochemistry Analysis Center of the third affiliated hospital of the Peking University Health Science Center (HITACHI 7170, Japan).

2.5. Immunohistochemistry

Immunohistochemical staining was performed for ghrelin and NPY using biotin–avidin–horseradish-peroxidase complex (ABC) methodology. The sections were incubated with ghrelin antibodies diluted 1:1000 (Phoenix Pharm., Belmont, CA, USA) and NPY antibodies diluted 1:4000 at 4 °C for 48 h; biotinylated goat anti-rabbit IgG diluted to 1:200 at 37 °C for 30 min; streptavidin–biotin-peroxidase complex diluted to 1:200, prepared 30 min before use, at 37 °C for 30 min (Vector ABC kit). 3,3′-Diaminobenzidine tetrahydrochloride (DAB, 0.05% in PBS, w/v) containing 0.005% (v/v) H_2O_2 was used as the substrate of HRP. Between these steps, sections were washed three times for 5 min each with 0.01 M phosphate buffered saline (PBS). Negative controls were carried out with similarly treated adjacent sections omitting the primary antibody, and no positive immunostaining was detected. After taking the digital photographs at 10× magnification under a light microscope (Olympus, China) with a digital camera (Leica Microsystems, Germany), the integral optical density of ghrelin and NPY-immunoreactivity were measured by a computerized image analysis program (Q-win, Leica Microsystems, Germany).

2.6. RT-PCR

The whole hypothalamus was homogenized in the presence of guanidinium isothiocyanate, and total RNA was extracted

using Trizol isolation kit (Invitrogen Corporation, Carlsbad, CA). First strand cDNA was synthesized from approximately 1 μg total RNA in a volume of 25 μl containing 200 U M-MLV reverse transcriptase (Invitrogen Corporation, Carlsbad, CA), RT buffer in the presence of 10 mM dNTPs, 30U RNase inhibitor, and 0.5 μg oligo dT as primers. The thermal cycler was programmed for 60 min at 42 °C and 10 min at 70 °C.

NPY, AgRP, UCP3 and β-actin specific sense and antisense oligonucleotide primers were designed based on the published cDNA sequences—NPY [34] primer sense: 5′-TAG GTA ACA AAC GAA TGG GG-3′, antisense: 5′-AGG ATG AGA TGA GAT GTG GG-3′, 351 bp; AgRP [19] primer sense: 5′-AGG GCA TCA GAA GGC CTG ACCA-3′, antisense: 5′-CTT GAA GAA GCG GCA GTA GCAC-3′, 214 bp; UCP3 [11] primer sense: 5′-GAA CCA TCG CCA GGG AAG AAG GAG TCAG-3′, antisense: 5′-GGG GGA GCG TTC ATG TAT CGG GTC TTTA-3′, 250 bp; β-actin primer sense: 5′-TTG TAA CCA ACT GGG ACG ATA TGG-3′, antisense: 5′-GAT CTT GAT CTT CAT GG TGC TAGG-3′, 764 bp. Primers for amplification of cDNA were synthesized by the Sangon company (China). One microliter of the first strand cDNA reaction was used for PCR amplification in the presence of 1 U Taq DNA polymerase (Invitrogen Corporation, Carlsbad, CA) in Taq buffer, 0.2 mM dNTPs and 1 μM of each primer. PCR was performed with the following conditions: NPY at 94 °C for 3 min, 94 °C for 45 s, 60 °C for 45 s, 72 °C for 45 s for 31 cycles; AgRP at 94 °C for 3 min, 94 °C for 60 s, 59 °C for 45 s, and 72 °C for 60 s for 34 cycles; UCP3 at 94 °C for 2 min, 94 °C for 30 s, 56 °C for 50 s, 72 °C for 50 s for 36 cycles; β-actin at 95 °C for 3 min, 94 °C for 45 s, 65 °C for 45 s, 72 °C for 45 s for 31 cycles. After amplification, the products were separated on a 1.5% agarose gel in the presence of ethidium bromide. The intensities of the bands were evaluated using an ultraviolet-light box imaging system (Hoefer, San Francisco, CA). The greyscales of the bands were measured by Total-lab Software. NPY, AgRP, UCP3 mRNA were normalized to β-actin mRNA level.

2.7. Western blot

Frozen muscle tissues were minced in TES buffer (10 mM Tris, pH 7.5, 1 mM EDTA, 250 mM sucrose) supplemented with the following protease inhibitors: 1 mM benzamidine, 4 μg/ml aprtinin, 1 μg/ml pepstatin, 2 μg/ml leupeptin. Minced tissues were carefully disrupted in a glass homogenizer. Unbroken tissue and nuclei were removed by centrifugation of the homogenate at $750 \times g$ for 10 min. The supernatant was centrifuged at $10{,}000 \times g$ for 20 min, and the mitochondrial pellet was resuspended in 1 ml of TES buffer. Mitochondria were submitted to another round of 10 min of centrifugation of 750 and $10{,}000 \times g$, respectively. Mitochondrial protein content was assayed by the bicinchoninic acid (BCA) method according to the manufacturer's protocol (Pierce, USA). Proteins were first separated on a 12.5% SDS-polyacrylamide and then transferred onto nitrocellulose membrane by liquid electroblotting (Bio-Rad, USA) for 60 min (100 V) in transfer buffer (12 mM Tris–HCl, pH 8.3, 96 mM glycine and 20% methanol). Non-specific binding was achieved by preincubating the membrane with TBS-T (tris-buffer saline containing 0.1% Tween 20) supplemented with 5% dried non-fat milk for 1 h at room temperature. Anti-UCP-3 goat polyclonal antibody (Phoenix Pharm, Belmont, CA, USA) was diluted 1:1000 (v/v) with TBS-T, 2% dried non-fat milk, and incubated overnight at 4 °C. After extensive washing with TBS-T, the membrane was incubated with biotinylated anti-goat IgG (Beijing Zhongshan Golden Bridge Biotechnology Co. Ltd., China) diluted 1:1000 (v/v) with TBS-T (5% dried milk) at RT for 1 h. After washing in TBS-T, membranes were incubated with avidin–biotin-peroxidase complex (Beijing Zhongshan Golden Bridge Biotechnology Co. Ltd.) diluted 1:500 (v/v) with TBS-T (5% dried milk) at RT for 30 min. Bound peroxidase-conjugated antibody was revealed with the enhanced chemiluminescence reagents kit (ECL, Amersham Pharmacia Biotech). Membranes were exposed for 3 min on Biomax MR Kodak film. Signals were quantified by Total-lab Software. UCP3 protein levels were normalized to β-actin protein.

2.8. Statistical analysis

Results are shown as mean ± S.E.M. Statistical comparisons between three respective experimental groups were performed with one-way ANOVA, followed by a Newman–Keuls comparison test. Statistical comparisons between two respective experimental groups were performed using the unpaired Student's *t*-test. $P < 0.05$ was considered significant.

3. Results

3.1. Body weight and food intake

In the first 3 days, body weight in all three FR groups decreased: a decrease from 254.8 ± 3.4 to 229.4 ± 2.7 g in the 2 Hz EA group, from 256.4 ± 4.7 to 230.4 ± 4.1 g in the restraint group and from 255.9 ± 3.4 to 232.5 ± 3.8 g in the control group, respectively. Starting from the fourth day to the 18th day, the body weight of rats in the 2 Hz EA group decreased continuously from 227.1 ± 2.9 to 218.0 ± 4.0 g, while that of the restraint group and control group increased from 230.4 ± 4.1 to 233.7 ± 4.3 g, and from 229.3 ± 3.5 to 239.6 ± 5.1 g, respectively. The ratio of the final body weight over the initial body weight decreased significantly in the 2 Hz group (−14.2 ± 1.3%, $n = 10$) compared with that of the restraint group (−8.3 ± 2.3%, $n = 10$, $P < 0.001$) and control group (−9.2 ± 1.5%, $n = 10$, $P < 0.001$) (Fig. 1A).

When food availability was restricted to 1 h per day, the average food consumption for 18 days of FR rats (11.4 ± 0.4 g, $n = 10$) reached a level of 39.5 ± 2% of ad lib-fed rats (29.3 ± 1.2 g, $n = 10$, $P < 0.001$) (Fig. 1C). The average food intake of the 2 Hz EA group (9.6 ± 0.3 g, $n = 10$) was significantly lower than that of the restraint group (11.2 ± 0.5 g, $n = 10$, $P < 0.05$) and control group (11.4 ± 0.4 g, $n = 10$, $P < 0.05$) (Fig. 1B).

3.2. Changes of peri-renal fat mass, plasma triglycerides and glucose

The weight of the peri-renal fat pad of rats in the 2 Hz EA group (0.26 ± 0.05 g, $n = 5$) seemed to be lower than that of rats in the restraint group (0.44 ± 0.12 g, $n = 5$) and control group (0.40 ± 0.04 g, $n = 5$), although the difference was not statistically significant.

Plasma triglyceride concentration did not reveal any difference among the experimental groups.

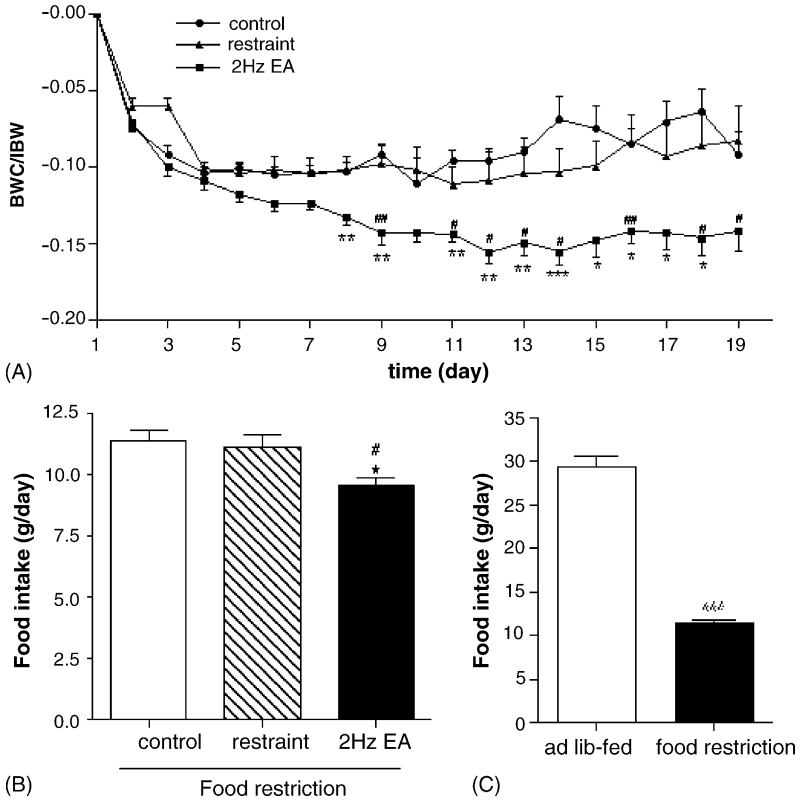

Fig. 1 – Effects of EA on body weight and food intake in FR rats. (A) The ratio of body weight change to initial body weight (BWC/IBW). (B) Average food intake of three FR groups. (C) Average food intake of ad-lid fed rats and FR rats. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. restraint group; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. control group; $^{\&\&\&}P < 0.001$ vs. ad-lid fed group.

Plasma glucose concentration of rats in the 2 Hz EA group (6.8 ± 0.2 mmol/l, $n = 5$) showed no significant difference compared with that of the control group (6.5 ± 0.2 mmol/l, $n = 5$), whereas that of the restraint group (7.8 ± 0.1 mmol/l) was significantly higher than the control group, $P < 0.01$ (Table 1).

3.3. *NPY and AgRP mRNA levels in hypothalamus*

RT-PCR studies revealed that NPY mRNA expression in the hypothalamus of the 2 Hz EA group (0.87 ± 0.03, $n = 5$) decreased significantly compared to that of the restraint group (0.98 ± 0.03, $n = 5$, $P < 0.05$) and control group (1.00 ± 0.02, $n = 5$, $P < 0.05$) (Fig. 2A and C). By contrast, the AgRP mRNA level did not reveal any differences among the experimental groups (Fig. 2A and D).

3.4. *NPY peptide levels in hypothalamus*

The integral optical density of NPY-immunoreactivities in PVN of the 2 Hz EA group (22.80 ± 3.65, $n = 5$) was significantly reduced compared with that of the restraint group (63.01 ± 8.61, $n = 5$, $P < 0.05$) and control group (100.0 ± 15.59, $n = 5$, $P < 0.001$), respectively (Fig. 2B and E).

3.5. *Ghrelin peptide expression in stomach*

The integral optical density of ghrelin immunoreactive cells in the stomach of the 2 Hz EA group (48.35 ± 8.741, $n = 5$) was significantly reduced compared with that of restraint group (113.0 ± 14.71, $n = 5$, $P < 0.01$) and control group (144.2 ± 12.99, $n = 5$, $P < 0.001$) (Fig. 3).

Table 1 – Changes of peri-renal fat mass, plasma triglycerides and glucose following EA treatment

Groups	Penrenal fat mass	Plasma glucose (mmol/l)	Plasma triglycerides (mmol/l)
Control ($n = 5$)	0.40 ± 0.04	6.5 ± 0.2	0.40 ± 0.06
Restraint ($n = 5$)	0.44 ± 0.12	$7.8 \pm 0.22^{**}$	0.43 ± 0.04
2 Hz EA ($n = 5$)	0.26 ± 0.05	$6.8 \pm 0.2^{\#}$	0.41 ± 0.05

** $P < 0.01$ vs. control.
$^{\#}$ $P < 0.05$ vs. restraint group.

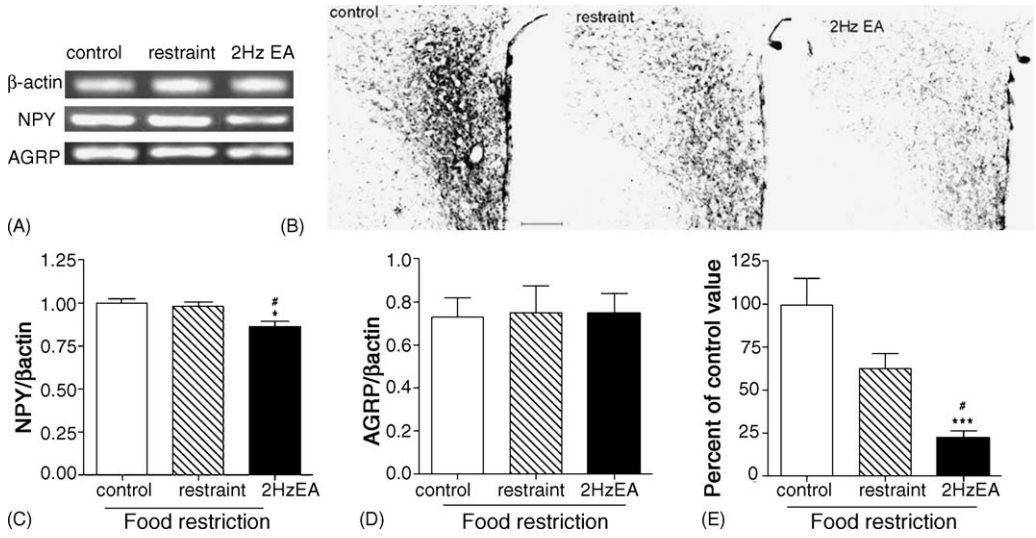

Fig. 2 – Hypothalamic NPY and AgRP levels in FR rats after EA treatment. (A) Representative image of relative quantitative RT-PCR analysis of hypothalamic NPY and AgRP mRNA with β-actin mRNA as an internal standard. (B) Photomicrographs of NPY-immunoreactivity in the PVN. (C and D) Quantification of NPY and AgRP mRNA normalized to β-actin mRNA. (E) The integral optical density of NPY-immunoreactivity in the PVN. $^{\#}P < 0.05$ vs. restrain group; $^{*}P < 0.05$, $^{***}P < 0.001$ vs. control group. Scale bar = 50 μm.

3.6. *UCP3 mRNA and protein levels in gastrocnemius*

RT-PCR analysis showed that UCP3 mRNA expression levels in the gastrocnemius in rats of the 2 Hz EA group (0.31 ± 0.01, $n = 5$) was not significantly different from that of the restraint group (0.33 ± 0.03, $n = 5$) or control group (0.35 ± 0.02, $n = 5$).

Western-blot analysis revealed that protein expression levels of UCP3 in the gastrocnemius in rats of the 2 Hz EA group (0.67 ± 0.09, $n = 5$) were slightly lower than that of the restraint group (0.82 ± 0.03, $n = 5$) and control group (0.86 ± 0.04, $n = 5$), although the differences were not statistically significant (Fig. 4).

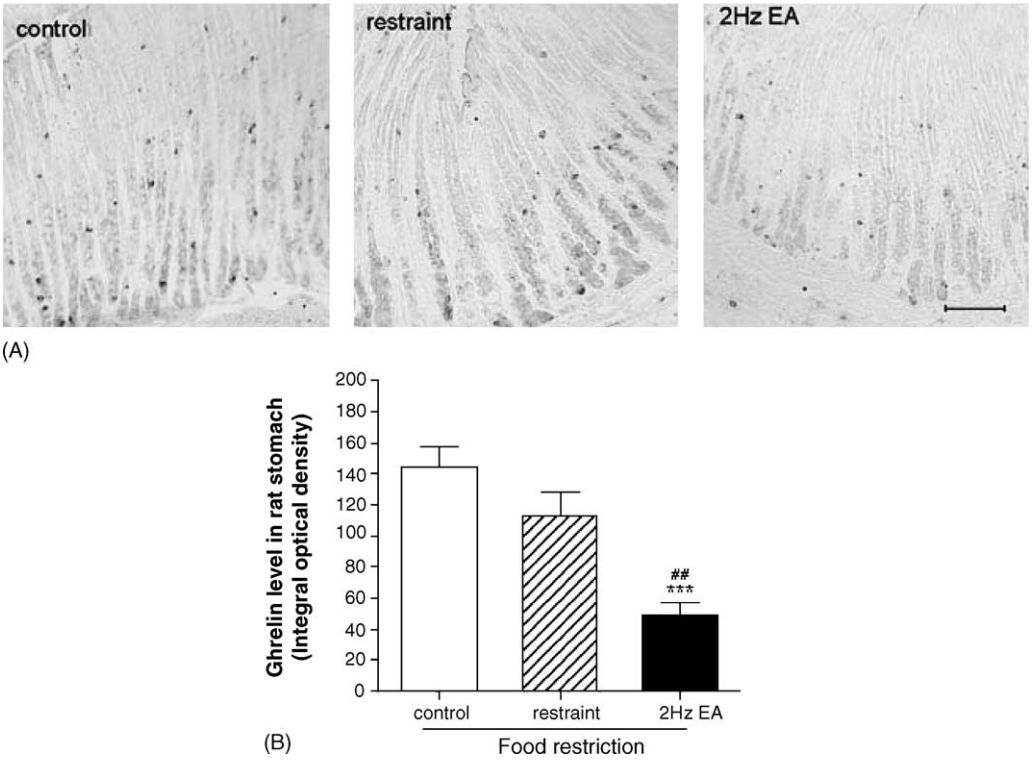

Fig. 3 – Stomach ghrelin levels in FR rats after EA treatment. (A) Photomicrographs illustrate the distribution of ghrelin-immunoreactive cells in stomach. (B) The integral optical density of ghrelin immunoreactive cells in stomach. $^{\#\#}P < 0.01$ vs. restraint group; $^{***}P < 0.001$ vs. control group. Scale bar = 50 μm.

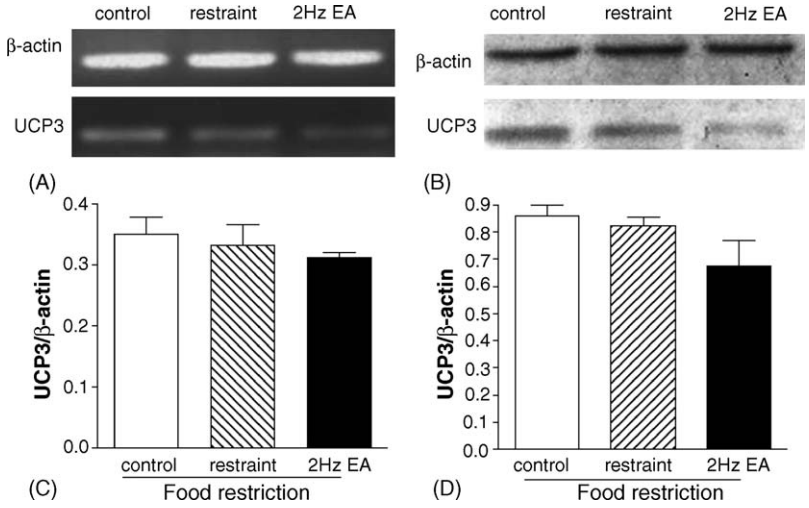

Fig. 4 – UCP3 expression in gastrocnemius of FR rats after EA treatment. (A and C) Representative image of relative quantitative RT-PCR analysis of UCP3 mRNA with β-actin mRNA as an internal standard. (B and D) Representative image of relative quantitative Western-blot analysis of UCP3 protein with β-actin as an internal standard.

4. Discussion

When access to food was restricted, animals showed a dramatic increase in the rate of food intake. In the present study, when food availability was restricted to only one hour per day, the average food consumption of FR rats reached a level of 39.5 ± 2% of that of ad lib-fed rats.

How do we decide when to eat and how much to eat? Brain-gut hormones are known to play dominant roles in appetite and short-term energy balance. A meal might be initiated by ghrelin signals and terminated by cholecystoknin (CCK), peptide YY (PYY) and glucagon-like peptide 1 (GLP-1) [23]. Stomach-derived ghrelin was the first identified peripheral orexigenic peptide. This peptide was initially found as an endogenous ligand of the growth hormone secretagogue receptor (GHSR) [17]. In addition to its ability to stimulate GH secretion, ghrelin was later found to stimulate caloric intake and increase body weight and adiposity [13]. One report revealed that 16 h of fasting significantly increased circulating ghrelin levels 2.1-fold in lean animals [24]. In the present study, we found that the expression of ghrelin peptides in the stomach was significantly upregulated after food restriction for 18 days (data not shown). Elevated ghrelin levels might contribute to increased food intake during starvation [14].

According to the prevalent hypothesis of Yin-Yang balance, the essence of acupuncture treatment is to redress the homeostasis that has become abnormally unbalanced in diseased conditions. Therefore, our prediction at the beginning of the study was that while EA lowered the body weight in DIO rats [30], it might increase the body weight in extremely lean rats. However, the results show a significant decrease in food intake in EA treated rats rather than an augmentation of food ingestion, resulting in a further reduction in body weight.

What are the possible mechanisms underlying this body weight reduction? One possibility was that since these rats were in a malnourished state, they might not have had sufficient physical strength to further increase the speed of eating. This is unlikely because rather than simply showing no increase in the speed of eating, rats treated with 2 Hz EA showed a significant decrease. In addition, EA treatment did indeed downregulate stomach ghrelin expression in FR rats. Other studies have shown that EA at ST 36 could elevate the secretion of somatostatin and CCK-8 [15,35], as both are potential antagonists of ghrelin [8,29]. Taken together, the decrease in stomach ghrelin could be regarded as an important link whereby 2 Hz EA exerted a body weight reduction effect.

Ghrelin in the brain was thought to exert its orexigenic action mainly via the arcuate nucleus (ARC) of the hypothalamus. C-Fos expression increased in ARC NPY-synthesizing neurons after peripheral administration of ghrelin [31]. In addition, ghrelin failed to increase food intake following ablation of the ARC [4]. Studies in knockout mice demonstrated that both NPY and AgRP signaling mediated the effects of ghrelin [4].

The ARC is an important site for the translation of diverse hormonal signals into behavioral and metabolic responses. Two orexigenic peptides, NPY and AgRP, are expressed in the same neurons in the medial ARC, whereas two anorexigenic peptides, pro-opiomelanocortin (POMC) and CART are co-expressed in the lateral ARC. Hypothalamic NPY, which is synthesized primarily in ARC neurons, projects to adjacent hypothalamic areas such as the paraventricular nucleus (PVN) and lateral hypothalamic area (LHA). NPY produces a shift to positive energy balance by increasing food intake and reducing energy expenditure [12]. Following 2 Hz EA treatment, NPY mRNA expression and peptide level in the hypothalamus decreased significantly in FR rats. The decreased ghrelin expression in the stomach might partly underlie this decline of NPY in the hypothalamus. Our previous work demonstrated that 2 Hz EA elevated levels of anorexigenic peptides such as CART [30] and α-MSH (unpublished data) in the hypothalamus in the diet-induced obesity model. Both the increase in anorexigenic peptides (CART, α-MSH) and the decrease in the orexigenic peptide NPY might contribute to the effects of EA on body weight reduction.

It is interesting to note that 2 Hz EA decreased the mRNA level of NPY, but not that of AgRP. It has been speculated that each of these two peptides might have its own optimal frequency for activation [9]. Further studies are necessary to measure the expression of AgRP mRNA expression following high-frequency (e.g., 100 Hz) EA.

The maintenance of the energy balance involves coordinated changes in energy intake and expenditure. It has been speculated that UCP3 affects energy metabolism in the muscle by utilizing fatty acids [26]. Several studies have suggested that UCP3 might serve as a new target responsible for body weight loss by upregulating energy expenditure [10,25]. In the present study, 2 Hz EA failed to induce over-expression of UCP3. On the contrary, UCP3 tended to decrease. Several findings have shown that sympathetic stimulation induced expression of UCP3 mRNA [22]. The possibility arises that 2 Hz EA might produce a suppression of sympathetic nerve activities, thereby masking the constitutive sympathetic activation [20,21].

It is interesting to note that the blood glucose level was significantly elevated in rats of the restraint group. This suggested that restraint in the holder did produce certain degree of stress. However, in the 2 Hz EA group this increase was eliminated. One might postulate that EA per se had an anti-stress effect via suppression of the sympathetically mediated glycolysis [3].

In summary, the effects of 2 Hz EA on body weight reduction might depend on decreasing food intake rather than increasing energy expenditure. The decreased expression of both ghrelin in the stomach and NPY in the hypothalamus following 2 Hz EA treatment might contribute to the reduced food intake in FR rats.

Acknowledgements

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