Peptides 32 (2011) 2394–2399

Contents lists available at SciVerse ScienceDirect

Peptides

journal homepage: www.elsevier.com/locate/peptides

Arcuate nucleus of hypothalamus is involved in mediating the satiety effect of electroacupuncture in obese rats

Fei Wang[a,c], De Run Tian[a,b,*], Patrick Tso[c], Ji Sheng Han[a]

[a] Neuroscience Research Institute, Peking University, Beijing 100083, PR China
[b] Department of Anatomy, Tianjin Medical University, Tianjin 300070, PR China
[c] Departments of Pathology and Laboratory Medicine and Psychiatry, University of Cincinnati, Cincinnati, OH, United States

ARTICLE INFO

Article history:
Received 18 August 2011
Received in revised form 19 October 2011
Accepted 20 October 2011
Available online 25 October 2011

Keywords:
Diet-induced obesity
Electroacupuncture
Arcuate nucleus of hypothalamus
POMC
α-MSH

ABSTRACT

Obesity is a major health problem in the world. Since effective remedies are rare, researchers are trying to discover new therapies for obesity, and acupuncture is among the most popular alternative approaches. This study investigated the anti-obesity mechanisms of EA, using a rat model of diet-induced obesity. After feeding with a high-fat diet for 9 weeks, a number of rats who gained weight that surpassed the maximal body weight of rats in the chow-fed group were considered obese and employed in the study. A 2 Hz EA treatment at the acupoints ST36/SP6 with the intensity increasing stepwise from 0.5–1–1.5 mA was given once a day for 30 min. Rats treated with EA showed significantly decreased food intake and reduced body weight compared with the rats in DIO and restraint group. EA treatment increased peptide levels of α-MSH and mRNA levels of its precursor POMC in the arcuate nuclear of hypothalamus (ARH) neurons. In addition, the cerebral spinal fluid (CSF) content of α-MSH was elevated by EA application. ARH lesions by monosodium glutamate abolished the inhibition effect of EA on food intake and body weight. A non-acupoint stimulation did not show the benefit effect on food intake inhibition and body weight reduction compared with restraint and ST36/SP6 EA treatment. We concluded that EA treatment at ST36/SP6 acted through ARH to significantly inhibit food intake and body weight gain when fed a high-fat diet and that the stimulation of α-MSH expression and release might be involved in the mechanism.

1. Introduction

Obesity is becoming a health threat all over the world in recent years. Individuals who are obese are at greater risk for suffering diabetes, hypertension, dyslipidemia, cardiovascular disease and sleep apnea [23]. Only one anti-obesity medication (Orlistat) is currently approved by the FDA for long-term use, and it is often along with side effects such as oily spotting bowel movements, oily stools, stomach pain, and flatulence [17]. Bariatric surgeries have been successively used for the long-term control of morbid obesity, but have potentials for perioperative complications and malnutrition [13].

Researchers are also interested in developing complementary and alternative approaches to treat obesity. Acupuncture, which has been practiced for thousands of years in Eastern countries, has been found effective to reduce body weight in both human and animal studies [15]. However, the precise mechanism is still unclear. Electroacupuncture (EA) is a modified acupuncture technique that utilizes electrical stimulation. The parameters of the EA can be precisely characterized and the results are more reproducible. Our previous studies have demonstrated the effect of EA on body weight reduction of obese rats [27].

In the present study, we employed the diet induced obese rats as the animal model, and treated them with EA. We revealed that POMC (α-MSH) participated in mediating the anorexigenic effect of EA and intact arcuate nuclear of hypothalamus (ARH) was required for the impact of EA on the suppression of food intake and body weight.

2. Material and method

2.1. Animals

Male Sprague-Dawley (SD) rats were obtained from Vital Company, Beijing. Animals were housed in a facility with a controlled temperature ($22 \pm 2\,^{\circ}C$) and maintained in 12/12 h light–dark cycles (light on from 07:00 to 19:00 h). To acclimatize to the new environment, all rats were fed with standard laboratory chow and water available *ad libitum* during the first week of the experiment. All procedures were performed in accordance with institutional

* Corresponding author at: Department of Anatomy, Tianjin Medical University, Tianjin 300070, PR China. Tel.: +86 22 2354 2724; fax: +86 22 2354 2724.
E-mail address: tiandr@tijmu.edu.cn (D.R. Tian).

0196-9781/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.peptides.2011.10.019

guidelines from the Animal Care Committee at Peking University. Animals were then randomly divided into two groups: (1) the control group ($n = 20$) was fed with standard laboratory chow (Vital Company Beijing); (2) the high fat (HF) group ($n = 100$) was fed with HF diet as described [26]. Body weight was monitored once every week. After feeding for nine weeks, rats in the HF group with body weights surpassed the maximum body weights of rats in the control group were assigned to be diet induced obese (DIO) rats.

2.2. EA treatment

DIO rats were divided into the following three groups: EA group, the restraint group and the DIO group, who received no further treatment serving as control. Rats of each group were housed individually and acclimated to the new environment for one week. Rats of EA and the restraint group were kept in plastic tubes during the EA treatment. Stainless steel acupuncture needles were soldered to a wire that was connected to one of the output channels of an electronic stimulator. (Han's Acupoint Nerve Stimulator (HANS), manufactured by Hua Wei Company, Beijing, China). Two needles were inserted into the points Zusanli (ST36, near the knee joint, 5 mm lateral to the anterior tubercle of the tibia) and 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) at each hind leg of the rats. The EA parameters were set as follows: constant current square wave output (pulse width, 0.6 ms at 2 Hz); intensities ranging from 0.5, 1.0 to 1.5 mA, with each intensity lasting for 10 min. EA experiment was performed in 18:00–19:00 pm. EA was given 30-min per session, seven times per week for two weeks. Rats in the restraint control group were restrained for 30 min without EA. In all groups, high fat diet were provided, food intake and body weight were measured daily.

2.3. Immunohistochemistry and in situ hybridization

The immunohistochemistry procedure was performed as described [26]. α-MSH antibody was obtained from Phoenix Pharm (Belmont, CA, USA). Signal was visualized using a Vectastain ABC kit (Vector). The α-MSH peptide-immunoreactive neurons were counted under 10× magnification. Counting was done in a blind manner as to the identity of the sample by a single observer. Six sections were counted for α-MSH peptide positive neurons per brain. Comparisons between groups were performed by analysis of five randomly assigned animals per group, and the cell count in each section was determined from both the left and right sides of the ARH. For *in situ* hybridization, digoxigenin-labeled antisense cRNA probe was generated from a plasmid containing the POMC gene (NM_139326, 57-438). Sections were washed in PBS, treated with proteinase K, fixed, acetylated, and hybridized overnight at 58 °C using 2 ng/mlcRNA probes. After hybridization, the slides were washed in 4× SSC containing 50% formamide for 30 min, incubated in RNase A (20 μg/ml) for 30 min at 37 °C, washed in 2× SSC, 0.1× SSC for 30 min at 37 °C, and then washed in PBS twice for 10 min. After immersed in 1.5% blocking reagent, sections were incubated with anti-Dig-AP antibody (1:1000) for 4 h at 37 °C, and then were washed in PBS for 10 min twice, buffer-1 (100 mM Tris–HCl, pH 7.5, 150 mM NaCl, 0.01% Tween 20) for 10 min and buffer-2 (100 mM Tris–HCl, pH 9.5, 100 mM NaCl, 50 mM $MgCl_2$) for 3 min. Finally, sections were stained using NBT/BCIP (400 μg/ml, 200 μg/ml). For control purposes, hybridization was also performed without a probe or in the presence of a sense probe. In control experiments, no staining was performed. Four sections from the arcuate nucleus of each animal spanning from −2.3 mm to −3.6 mm relative to Bregma were analyzed.

2.4. Measurement of CSF level of α-MSH

Right after a 30-min EA stimulation or restraint, the DIO rats were flexed downward of the head at approximately 45° under anesthesia. A small incision was made in the skin and the remaining tissue was removed to clearly expose the atlanto-occipital membrane between the occipital bone and the upper cervical vertebra. A butterfly needle connected with 200 μl of syringe was used to directly puncture into the cisterna magna, and about 100 μl sample was drawn into the syringe. Then the clear CSF is transferred into Eppendorf tubes and stored at −20 °C. α-MSH level in CSF was then measured using a commercial RIA kit according to the manufacturer's guide (Phoenix, CA).

2.5. Lesion of ARH by monosodium glutamate (MSG)

To produce ARH lesions, late-term pregnant female rats were allowed to deliver normally, pups were injected with MSG (4 mg/gm, i.p.; Sigma, St. Louis, MO) or saline every other day for the first 10 days postnatally. At 21 days of age, pups were separated from the mother and grouped on the basis of treatment status (lesion vs. control). At 16 weeks, rats underwent lesion of ARH were divided into two groups and treated either with EA or restraint once daily for one week. Body weight and food intake were measured every day. At the end of the study, serial coronal sections were prepared at ARH level to verify the ablation of ARH.

2.6. Non-acupoint EA treatment

The rats in the non-acupoint-EA group were subject to EA stimulation at a non-acupoint, which is located on the proximal part of the tail [12]. EA was given 30-min per session, seven times per week for two weeks. EA parameters were the same with ST36/SP6 EA group. Food intake and body weight were measured daily.

2.7. Statistical analysis

All data are expressed as mean ± SEM. Statistical differences among groups were determined using students' *t*-test, one-way ANOVA or two-way ANOVA. For all analyses, a $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Effects of EA on body weight and food intake in DIO rats

After two-week EA treatment, there was a significant decrease of the average body weight of the rats in EA group (−3.5%). At the same time, the average body weight of the rats in the restraint group and the DIO group were increased for 0.1% and 3.7%, respectively. The differences in changes of body weight between the EA treatment groups and the restraint or DIO group were significant ($p < 0.001$) (Fig. 1A, two-way ANOVA). During the same period, a lower food intake per day was observed in the EA group as compared to the DIO ($p < 0.001$) and restraint group ($p < 0.05$) (Fig. 1B, one-way ANOVA). Food intake of the restraint group was not statistically different with that of the DIO group.

3.2. Effect of EA on POMC (α-MSH) expression in DIO rats

After two-week of EA treatment, brain sections in all groups at the level of ARH were analyzed to determine the mRNA expression of POMC and peptide level of α-MSH. EA treated rats had higher level of POMC mRNA compared to DIO rats ($p < 0.05$) (Fig. 2, one-way ANOVA). EA treated rats also had more α-MSH positive neurons in the ARH compared with DIO ($p < 0.01$) and restraint

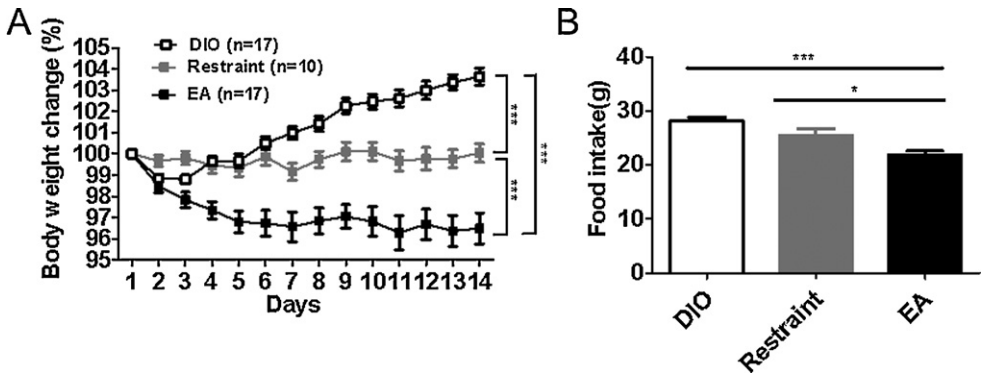


Fig. 1. (A) Daily body weight change compared to initial body weight of the rats in DIO, Restraint and EA group for 2 weeks. (B) Average daily food intake (g) of the rats in all groups. Data are given as the mean ± S.E.M. $^{*}p < 0.05$, $^{***}p < 0.001$.

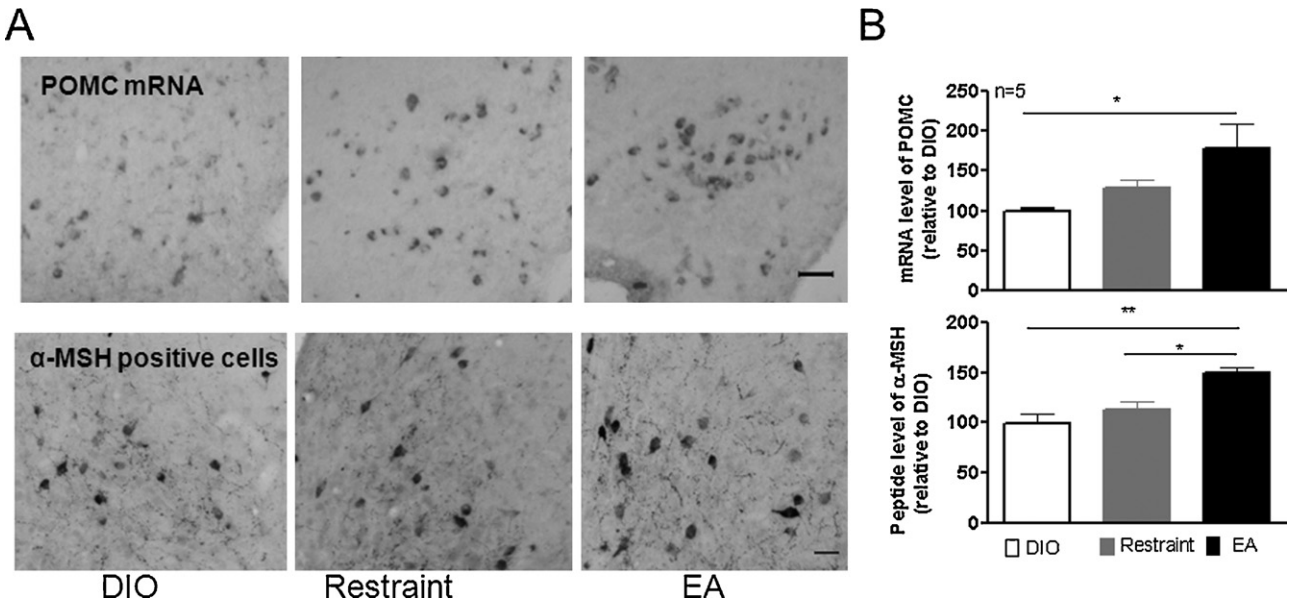


Fig. 2. (A) Bright-field photomicrographs of the ARH, showing POMC mRNA (upper) and α-MSH peptide (lower) expression. Scale bar = 50 μm. (B) Quantization of POMC mRNA and α-MSH peptide levels by densitometry shown in A. $n = 5$, $^{*}p < 0.05$, $^{**}p < 0.01$.

group ($p < 0.05$) (Fig. 2). Neither POMC nor α-MSH level was different between DIO and restraint group (Fig. 2).

3.3. Effect of EA on the level of α-MSH in CSF

α-MSH concentrations in CSF were measured after a 30-min restraint or EA treatment. The result showed that EA stimulation significantly elevated α-MSH level in CSF compared to restraint group ($p < 0.05$) (Fig. 3, *t*-test), suggesting an increased secretion from the producing neurons.

3.4. Effect of EA on body weight and food intake in ARH lesioned rats

Lesioned rats at the time of testing (16 weeks old) had a normal weight, but exhibited obviously stunted linear growth relative to control animals. Overt adiposity of lesioned rats was manifest as confirmed by higher Lee's Index ($BW/Length^2$) (Fig. 4B, *t*-test). Furthermore, examination of Nissl-stained series of sections from MSG-treated rats revealed that all lesioned animals displayed an enlarged third ventricle, as well as a profound cell loss in the ARH (Fig. 4A). The lesioned rats were randomly divided into two groups, and treated once a day with EA or restraint for one week. Average daily food intake and body weight change were not different between the two groups (Fig. 5).

3.5. Effect of non-acupoint EA on body weight and food intake in DIO rats

During the two weeks of experimental period, body weight changes of the rats in non-acupoint EA group were similar to that

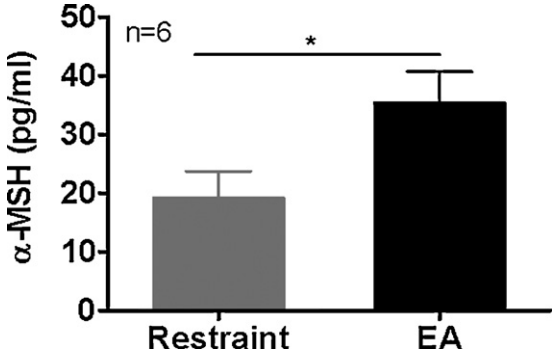
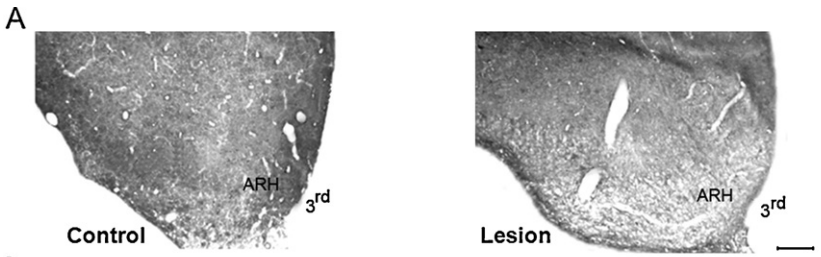


Fig. 3. CSF levels of α-MSH with or without EA stimulation. $n = 6$, $^{*}p < 0.05$.



B

Groups	Age (wks)	Body weight (g)	Length (cm)	Lee's index
Lesion	16	466.2±12.37	21.3±0.54***	1.03±0.03***
Control	16	468.2±19.68	25.1±0.33	0.75±0.02

Fig. 4. Evaluation of MSG lesions. (A) Sections through the ARH of representative control (*left*) and MSG-lesioned (*right*) rats. Examination of Nissl-stained material revealed substantial cell loss in the ARH of MSG-lesioned rats. 3rd, the third ventricular, scale bar = 50 μm. (B) Characteristics of MSG-lesioned rats were assessed by bodyweight, body length and Lee's index. $n = 6$, $^{***}p < 0.001$.

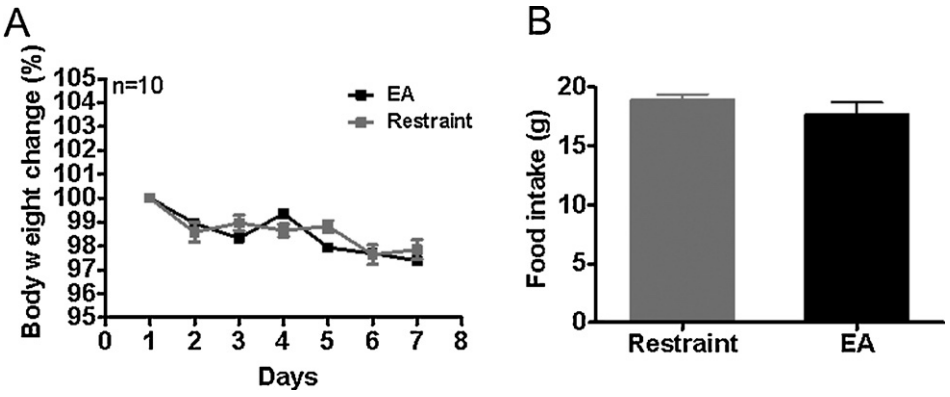


Fig. 5. Evaluation of the effect of EA on bodyweight (A) and food intake (B) in MSG-lesioned rats.

of restraint group (Fig. 6A, two-way ANOVA). The differences in changes of body weight between the ST36/SP6 EA group and non-acupoint EA group became significant since the sixth day ($p < 0.001$) (Fig. 6A). The average daily food intake was significantly lower in ST36/SP6 2 Hz group as compared to non-acupoint EA group ($p < 0.01$) (Fig. 6B, one-way ANOVA). There is no significant difference in food intake between the non-acupoint EA group and restraint or DIO group (Fig. 6B).

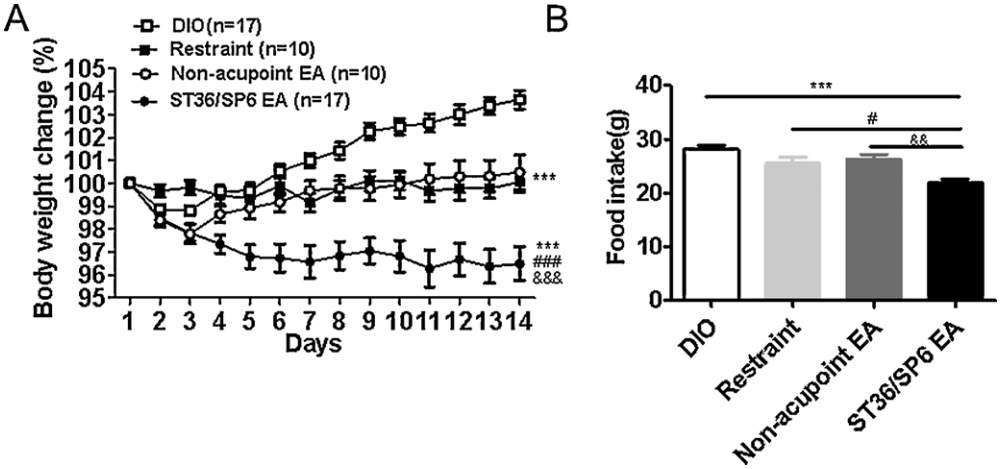


Fig. 6. Comparison of the effect of ST36/SP6 and non-acupoint EA on bodyweight (A) and food intake (B). $^{***}p < 0.001$ vs. DIO; $^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$ vs. restraint; $^{\&\&}p < 0.01$, $^{\&\&\&}p < 0.001$ vs. non-acupoint EA.

4. Discussion

Several clinical studies reported that successive EA stimulation reduced body weight and body mass index in obese subjects, although the effects were observed variable [4,11,29]. In our previous and the present study, we observed consistent effect of EA on reducing body weight of obese rats [27]. Most importantly, it was found that the body weight reduction was associated with the suppression of food intake, suggesting a central effect of EA (Fig. 1).

However, what kind of neurons or peptides mediated the effect of EA is unclear. In the ARC, the predominant anorexigenic peptide α-MSH, derived from the precursor POMC, bind to downstream MC4-Rs to inhibit food intake [6,7]. Consistent with this, knockout mice lacking the POMC gene display increased food intake and weight gain [30], while intracerebroventricular (ICV) administration of α-MSH to rats reduced food intake [21]. MC4-R mutations account for approximately 6% of severe early onset human obesity and 90 different mutations have been identified to be associated with obesity [8]. It was demonstrated that in obese rats with hyperphagia, the expression of POMC mRNA and α-MSH peptide in ARH was significantly decreased [18,26]. Intriguingly, we found in this study that EA stimulation significantly increased POMC mRNA and α-MSH peptide expression in the ARH of obese rats (Fig. 2). More importantly, we found that 30-min EA stimulation is sufficient to increase the level of α-MSH in CSF (Fig. 3), which suggested that more α-MSH was release from the POMC neurons after EA treatment. Since the ARH is one of the predominant regions to express the POMC gene in the brain, we speculate that the release of α-MSH from ARH POMC neurons accounts for at least part of the elevated level of CSF α-MSH. The released α-MSH could possibly act through the MC4 receptor on the target neurons and exert anorexigenic effect. We need to point out that POMC was also the precursor of many other biologically active peptides in addition to α-MSH [19]. Whether these peptides are regulated by EA in obese rats is unknown.

Our previous study indicated that EA stimulated CART peptide expression in the ARH as well. CART was reported as a hypothalamic satiety factor [14]. The majority of CART neurons in the ARC also contains POMC mRNA [28]. Animal studies have shown that ICV administration of CART inhibits food intake [14], whereas ICV injection of CART antibody increases food intake [14]. These observations indicated that both increased POMC (α-MSH) and CART expression could contribute to the anorexigenic effect of EA.

The ARH is a key hypothalamic nucleus in the regulation of appetite. It is well established that the ARH neurons integrate a number of peripheral signals controlling food intake, such as leptin, insulin and ghrelin [20,25]. Neuronal projections from ARH neurons also innervate other hypothalamic areas such as paraventricular nucleus (PVH), the dorsomedial nucleus (DMH), and the lateral hypothalamic area (LHA), each of which have been implicated in the regulation of appetite. [2]. Previous study reported that 2 Hz EA significantly stimulated c-fos expression ARH [10], implying the activation of ARH neurons. In this study, when the ARC was ablated by monosodium glutamate, EA lost its effect on food intake inhibition, and the body weight reduction was also abolished (Fig. 4). These observations suggest that intact ARH is required for the satiety effect of EA.

Besides the mechanism suggested here, other factors may contribute to the satiety effects of EA stimulation. A recent study reported that EA stimulation at Zusanli (ST36) led to decreased food intake in 48 h-fasting SD rats [12]. This effect was blocked by the pre-treatment with a CCK-1 receptor antagonist or in the CCK-1 receptor gene knockout OLETF rats. This research team strongly suggested that endogenous CCK acting through CCK-1 receptors played an important role in mediating the satiety effects of EA stimulation at ST36. Since CCK was known to activate the vagal afferent fibers through CCK-1 receptors [16,24], they investigated the effects of ST36 EA stimulation on food intake in the vagotomized rats and showed that subdiaphragmaticvagotomy reversed the satiety effects of ST36 EA stimulation, supporting the involvement of CCK acting peripherally in the EA stimulation-induced satiety. It is well accepted that vagal afferent neurons transmitted satiety signals from peripheral CCK to the nucleus of the solitary tract (NTS), where the satiety information communicated with descending hypothalamic inputs involved in food regulation [9,22]. These data and our findings in the present study suggest that both intact ARH signals and endogenous CCK pathway may be necessary for the satiety effect of EA stimulation. It was reported that NTS and hypothalamic nuclei communicated with each other and regulated feeding behavior [1]. Therefore, further studies regarding the possible interplay between CCK signal and ARH peptides could provide more evidences for applying peripheral EA stimulation in the regulation of food intake.

We chose ST36 and SP6 as acupoints in this study based on the following reasons. First, our previous study showed that EA treatment on these acupoints significantly reduced food intake and body weight [27]. Second, these two acupoints are commonly used by others to treat obesity either in animal studies or in clinical studies [5,12]. Third, stimulation of these acupoints was reported to increase c-fos expression in some central areas associated with appetite regulation, such as ARH and NTS [10]. To confirm the specificity of these acupoints, a group of rats were subject to EA stimulation at a non-acupoint, which is located on the proximal part of the tail. The non-acupoint stimulation did not produce additional body weight reduction or food intake inhibition as compared to the restraint group (Fig. 6). However, there were some other acupoints used for the treatment of obesity [5] which we did not investigate in this study. Future studies should be carried out to determine whether stimulation of other acupoints acts through the similar mechanism to impact food intake and body weight regulation as the stimulation of ST36/SP6.

A limitation of this study is that we assume the inhibition of food intake contributes to the reduction of body weight induced by EA treatment. But we are not certain whether food intake suppression is the only cause of body weight reduction. Body weight regulation is the balance of energy intake and energy expenditure. Whether EA also regulates energy expenditure is unknown. It was reported that EA therapy in obese women reduced serum total cholesterol, triglycerides, and LDL cholesterol levels [3]. This observation suggested a direct or indirect role of EA in mobilizing energy stores. In the future study it is interesting to involve a pair feeding group in the design of the experiment to clarify whether EA can regulate body weight partially independent of food intake, presumably by modulating energy storage in the periphery.

In summary, EA stimulation significantly reduced body weight gain, which is associated with the inhibition of food intake. EA stimulation increased peptide levels of α-MSH and mRNA levels of its precursor POMC in the ARH neurons. Moreover, the CSF content of α-MSH was increased by EA application. Lesions of ARH by glutamate abolished the inhibition effect of EA on food intake and body weight. The study suggests that ARH plays an important role in mediating the satiety effect of EA stimulation.

Funding

This research was supported by the Natural Science Foundation of Tianjin (07JCZDJC08100) to DRT, National Natural Science Foundation of China (30870791 to D.R. Tian) and a grant from the Ministry of Education (985 project) to JSH.

Disclosure statement

The authors have nothing to disclose.

References

[1] Blevins JE, Baskin DG. Hypothalamic–brainstem circuits controlling eating. Forum Nutr 2010;63:133–40.
[2] Bouret SG, Draper SJ, Simerly RB. Formation of projection pathways from the arcuate nucleus of the hypothalamus to hypothalamic regions implicated in the neural control of feeding behavior in mice. J Neurosci 2004;24:2797–805.
[3] 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.
[4] Cabioglu MT, Ergene N. Changes in levels of serum insulin, C-peptide and glucose after electroacupuncture and diet therapy in obese women. Am J Chin Med 2006;34:367–76.
[5] Cho SH, Lee JS, Thabane L, Lee J. Acupuncture for obesity: a systematic review and meta-analysis. Int J Obes (Lond) 2009;33:183–96.
[6] Cone RD. Studies on the physiological functions of the melanocortin system. Endocr Rev 2006;27:736–49.
[7] Fan W, Boston BA, Kesterson RA, Hruby VJ, Cone RD. Role of melanocortinergic neurons in feeding and the agouti obesity syndrome. Nature 1997;385:165–8.
[8] Farooqi IS, Keogh JM, Yeo GS, Lank EJ, Cheetham T, O'Rahilly S. Clinical spectrum of obesity and mutations in the melanocortin 4 receptor gene. N Engl J Med 2003;348:1085–95.
[9] Glatzle J, Kreis ME, Kawano K, Raybould HE, Zittel TT. Postprandial neuronal activation in the nucleus of the solitary tract is partly mediated by CCK-A receptors. Am J Physiol Regul Integr Comp Physiol 2001;281:R222–9.
[10] Guo HF, Tian J, Wang X, Fang Y, Hou Y, Han J. Brain substrates activated by electroacupuncture of different frequencies (I): comparative study on the expression of oncogene c-fos and genes coding for three opioid peptides. Brain Res Mol Brain Res 1996;43:157–66.
[11] Hsu CH, Hwang KC, Chao CL, Lin JG, Kao ST, Chou P. Effects of electroacupuncture in reducing weight and waist circumference in obese women: a randomized crossover trial. Int J Obes (Lond) 2005;29:1379–84.
[12] Kim SK, Bae H, Lee G, Jeong H, Woo HS, Han JB, et al. The endogenous CCK mediation of electroacupuncture stimulation-induced satiety in rats. Peptides 2008;29:564–70.
[13] Korenkov M, Sauerland S, Junginger T. Surgery for obesity. Curr Opin Gastroenterol 2005;21:679–83.
[14] Kristensen P, Judge ME, Thim L, Ribel U, Christjansen KN, Wulff BS, et al. Hypothalamic CART is a new anorectic peptide regulated by leptin. Nature 1998;393:72–6.
[15] Lacey JM, Tershakovec AM, Foster GD. Acupuncture for the treatment of obesity: a review of the evidence. Int J Obes Relat Metab Disord 2003;27:419–27.
[16] Lal S, Kirkup AJ, Brunsden AM, Thompson DG, Grundy D. Vagal afferent responses to fatty acids of different chain length in the rat. Am J Physiol Gastrointest Liver Physiol 2001;281:G907–15.
[17] Li MF, Cheung BM. Rise and fall of anti-obesity drugs. World J Diabetes 2011;2:19–23.
[18] Lin S, Storlien LH, Huang XF. Leptin recepto, NPY, POMC mRNA expression in the diet-induced obese mouse brain. Brain Res 2000;875:89–95.
[19] Millington GW. The role of proopiomelanocortin (POMC) neurones in feeding behaviour. Nutr Metab (Lond) 2007;4:18.
[20] Peruzzo B, Pastor FE, Blazquez JL, Schobitz K, Pelaez B, Amat P, et al. A second look at the barriers of the medial basal hypothalamus. Exp Brain Res 2000;132:10–26.
[21] Rossi M, Kim MS, Morgan DG, Small CJ, Edwards CM, Sunter D, et al. A C-terminal fragment of Agouti-related protein increases feeding and antagonizes the effect of alpha-melanocyte stimulating hormone in vivo. Endocrinology 1998;139:4428–31.
[22] Schwartz MW, Woods SC, Porte Jr D, Seeley RJ, Baskin DG. Central nervous system control of food intake. Nature 2000;404:661–71.
[23] Sha H, He Y, Yang L, Qi L. Stressed out about obesity: IRE1alpha-XBP1 in metabolic disorders. Trends Endocrinol Metab 2011.
[24] 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.
[25] Stanley S, Wynne K, McGowan B, Bloom S. Hormonal regulation of food intake. Physiol Rev 2005;85:1131–58.
[26] Tian DR, Li XD, Shi YS, Wan Y, Wang XM, Chang JK, et al. Changes of hypothalamic alpha-MSH and CART peptide expression in diet-induced obese rats. Peptides 2004;25:2147–53.
[27] Tian DR, Li XD, Wang F, Niu DB, He QH, Li YS, et al. Up-regulation of the expression of cocaine and amphetamine-regulated transcript peptide by electroacupuncture in the arcuate nucleus of diet-induced obese rats. Neurosci Lett 2005;383:17–21.
[28] Vrang N. Anatomy of hypothalamic CART neurons. Peptides 2006;27:1970–80.
[29] Wang F, Tian DR, Han JS. Electroacupuncture in the treatment of obesity. Neurochem Res 2008;33:2023–7.
[30] Yaswen L, Diehl N, Brennan MB, Hochgeschwender U. Obesity in the mouse model of pro-opiomelanocortin deficiency responds to peripheral melanocortin. Nat Med 1999;5:1066–70.