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The association of serum leptin with the reduction of food intake and body weight during electroacupuncture in rats

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Abstract

Previous studies indicate that acupuncture or electroacupuncture (EA) treatment reduces body weight and food intake in rats by increasing the level of anoretic peptides and decreasing that of orexigenic peptides in the hypothalamus. Considering a well-established role of leptin as a major regulator for feeding behavior in the hypothalamus, we hypothesized that EA might exert its effect via increasing serum leptin levels. In this study, we tested our hypothesis by evaluating the effects of EA on food intake and body weight, as well as on serum leptin levels in rats. Rats were randomly divided into 3 groups: AL (fed ad libitum with no treatments), Holder (fed ad libitum with daily holder restraint) and EA (fed ad libitum with daily holder restraint and 100 Hz EA stimulation) groups. During the four-week experimental period, daily food intake and body weight were measured. At the end of the experiment, levels of serum leptin and corticosterone, and plasma epinephrine (Epi) and norepinephrine (NE) were determined. Here we demonstrate that EA treatment indeed led to reduction of food intake and body weight, and to an increase of serum leptin levels. The level of Epi, NE, and corticosterone increased in the Holder group, but such increase in the level of aforementioned stress hormones was not observed in the EA group. Overall, our results suggest that EA treatment reduces food intake and body weight in rats possibly through increasing leptin levels, and that this effect of EA is not due to the stress caused by the daily holder restraint.

Keywords: Electroacupuncture; Food intake; Body weight; Leptin; Rat

1. Introduction

The body weight control systems appear to respond more sensitively to weight loss rather than weight gain. This notion has been reflected in human evolutionary history, in which food scarcity, not overabundance, was the major danger. However, most people in developed and developing countries now have access to an abundance of food and tend to become more sedentary (Friedman, 2003; Marx, 2003; Pi-Sunyer, 2003). Obesity, which is considered a major risk factor for life-threatening diseases including type II diabetes, heart attack, stroke, and some types of cancer, has been increasing at an alarming rate in recent years, becoming now a worldwide public health problem (Friedman, 2000; Marx, 2003; Lazar, 2005). Increases in adult prevalence of obesity are reflected by a striking increase in childhood and adolescent weight. The early onset of obesity leads to an increased likelihood of obesity in later life as well as an increased prevalence of obesity-related diseases (Dietz, 1994; Kotani et al., 1997; Kopleman, 2000; Lazar, 2005). Therefore, proper regulation of food intake and body weight before adulthood is required.

Acupuncture has long been used in Eastern countries for the treatment of various diseases, generating few side effects, and is recently considered a new alternative method of medicine in Western countries (NIH Consensus Conference, 1998; Kaptchuk, 2002; Lacey et al., 2003). Previous studies have shown that acupuncture treatment increases the activity of the ventromedial

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hypothalamus (VMH, "satiety" center) in obese rats (Zhao et al., 2000) and decreases neuropeptide Y (NPY) expression in the arcuate nucleus of diabetic rats (Lee et al., 2004). Electroacupuncture (EA) is a modified technique of acupuncture using electrical stimulation and was found to have beneficial effects on obesity in children (Gadzhiev et al., 1993). Recently, it has been reported that low and high frequency EA significantly decreased food intake and body weight, and up-regulated the expression of α-melanocyte-stimulating hormone (α-MSH) and cocaine- and amphetamine-regulated transcript (CART) in the hypothalamic arcuate nucleus of obese rats (Tian et al., 2003, 2005).

The hormone leptin, released into the bloodstream by adipocytes, regulates feeding behavior by acting directly on neurons of the hypothalamus. A decrease in body fat leads to a decreased level of leptin, which in turn stimulates food intake by increasing NPY and agouti-related protein (AgRP) in the arcuate nucleus. On the other hand, increased body fat is associated with increased leptin levels, which act to reduce food intake by increasing α-MSH and CART in the arcuate nucleus (Schwartz et al., 2000; Bear et al., 2001; Ahima and Osei, 2004).

Based on the similarity between the physiological results of leptin action and the central effects of EA on food intake and body weight, we hypothesized that EA might reduce food intake and body weight by increasing the serum leptin levels. To explore our hypothesis, we examined the effects of EA on food intake and body weight, and on serum leptin in young male Sprague–Dawley (SD) rats.

2. Materials and methods

2.1. Experimental animals

Young male SD rats (Sam:TacN(SD)BR, 190–210 g, 7 weeks) were housed in groups of four, with water and food available ad libitum. The room was maintained with a 12-h light/dark cycle (08:00–20:00 light, 20:00–08:00 dark) and kept at 23±2 °C. After 5-day acclimation in this laboratory environment, rats were randomly divided into 3 groups: AL group (fed ad libitum with no treatments, $n=8$, 4 rats/cage), Holder group (fed ad libitum with daily holder restraint for 4 weeks, $n=8$, 4 rats/cage) and EA group (fed ad libitum with daily holder restraint and high frequency EA stimulation at Zusanli (ST36) acupoint for 4 weeks, $n=8$, 4 rats/cage). Daily food intake and body weight were measured at 14:00 every day during the 4-week experimental period. The study was approved by the Institutional Animal Care and Use Committee of Kyung-Hee University and all procedures were conducted in accordance with the NIH guidelines.

2.2. EA stimulation

Rats of the Holder and EA groups were restrained in a plastic holder (5.3×15, 5.6×17, 6.0×24 cm in diameter×length). Two stainless-steel needles of 0.25 mm in diameter and 4 cm in length were inserted into the Zusanli (ST36) acupoint which is located at the anterior tibial muscle and about 10 mm below the knee joint (Fig. 1). This point is known to reduce body weight and food intake in rats (Tian et al., 2003, 2005). For EA stimulation, train-pulses (100 Hz, 0.3 ms pulse width, 0.2–0.3 mA) were applied to the inserted needle for 30 min. The other needle (anode) was inserted into the anterior tibial muscle 5 mm distal to the first one. Anodal and cathodal leads from an electrical stimulator were connected to the two acupuncture needles. The rat in the Holder group was restrained for 30 min without EA stimulation. The EA stimulation and holder restraint experiment were performed between 14:00 and 15:00.

2.3. Measurement of serum leptin and corticosterone, and plasma catecholamines

On the final day of the experiments, cardiac blood was collected under CO_2 asphyxiation. Serum and plasma were separated, respectively, and stored at −70 °C until the day of the analysis. Serum leptin levels were determined with commercially available $[I]^{125}$-labelled rat leptin RIA kits (Linco Research, Inc., USA). The measurement of serum corticosterone was performed with a commercial rat corticosterone EIA kits (DSL, USA). Plasma Epi and NE were assayed using HPLC with electrochemical detection.

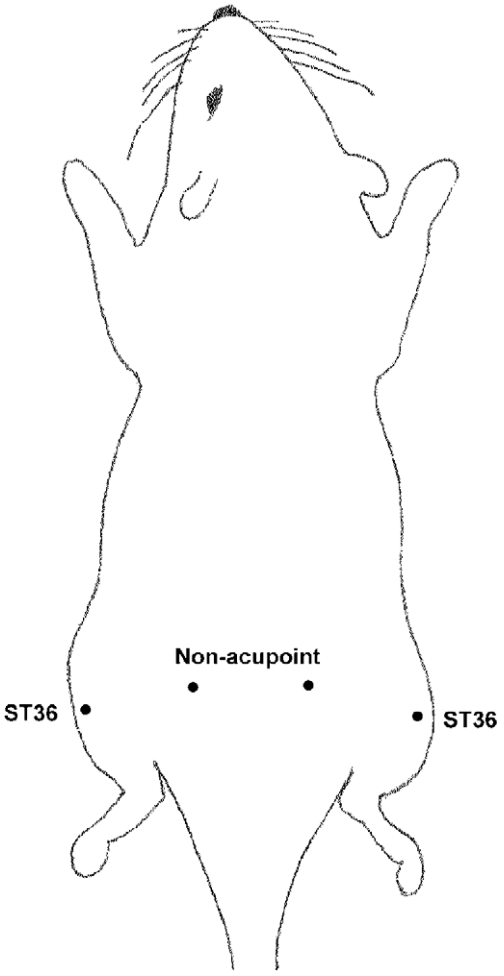

Fig. 1. Location of the acupoint ST36 and non-acupoint in the rat. ST36=Zusanli acupoint which is located at the anterior tibial muscle and about 10 mm below the knee joint. Non-acupoint=located midway between the coccyx and hip joints.

2.4. Statistical analysis

Data are presented as mean±SEM. For the statistical analysis, the one-way analysis of variance (ANOVA) followed by Newman–Keuls multiple comparison test was used. In all cases, $p<0.05$ was considered significant.

3. Results

3.1. Effects of EA on food intake and body weight

The effects of EA on food intake and body weight in rats are shown in Table 1 and Fig. 2. Total food intake of rats in the EA group during the 4-week experimental period was significantly reduced ($p<0.05$, AL or Holder group vs EA group) (Table 1). Daily food intake in the EA group was also reduced by approximately 10~20%, when compared with the Holder or AL group (Fig. 2a). Total body weight gain of rats in the EA group during the 4-week experimental period was significantly reduced ($p<0.001$, AL or Holder group vs EA group). In rats of the Holder group, the total body weight gain was also significantly decreased when compared with the AL group ($p<0.01$) (Table 1). There were marked differences in daily body weight change among the three groups (Fig. 2b). The body weight of rats in the EA group on the final experimental day was significantly low, compared with the AL or Holder group ($p<0.001$, AL group vs EA group; $p<0.01$, Holder group vs EA group). In rats of the Holder group, the body weight on the final experimental day was also significantly low, compared with the AL group ($p<0.01$). For the active control of the EA group, additional rats ($n=8$) were fed ad libitum with daily holder restraint and high frequency EA stimulation at "non-acupoint", which is located midway between the coccyx and hip joints (Fig. 1), for 4 weeks. However, their total food intake and body weight gain was not significantly different from that in the Holder group (Table 1).

3.2. Effects of EA on serum leptin levels

The effects of EA on serum leptin levels are shown in Fig. 3. In the EA group, the levels of serum leptin were apparently increased, showing statistically significant differences in serum leptin levels between AL or Holder and EA groups ($p<0.05$, AL or Holder group vs EA group). However, there were no significant differences in the leptin levels between the Holder and AL group ($p>0.05$).

Table 1
Total food intake and body weight gain during the 4-week experimental period

	AL	Holder	Non-Acu	EA
Total food intake (g)	575±12.5	544.9±0.9	534.5±11.25	490.1±11.6*,#,†
Body weight gain (g)	119.5±5.0	96.6±4.3**	91.2±2.7**	67.6±5.5***,###,††

Data are presented as mean±SEM. $N=8$ rats/group. *$p<0.05$, **$p<0.01$, ***$p<0.001$, vs AL; #$p<0.05$, ###$p<0.001$, vs Holder; †$p<0.05$, ††$p<0.01$, vs Non-Acu by Newman–Keuls multiple comparison test after one-way ANOVA. Non-Acu = Rats which were fed ad libitum with daily holder restraint and high frequency EA stimulation at "non-acupoint" during 4-week experimental period.

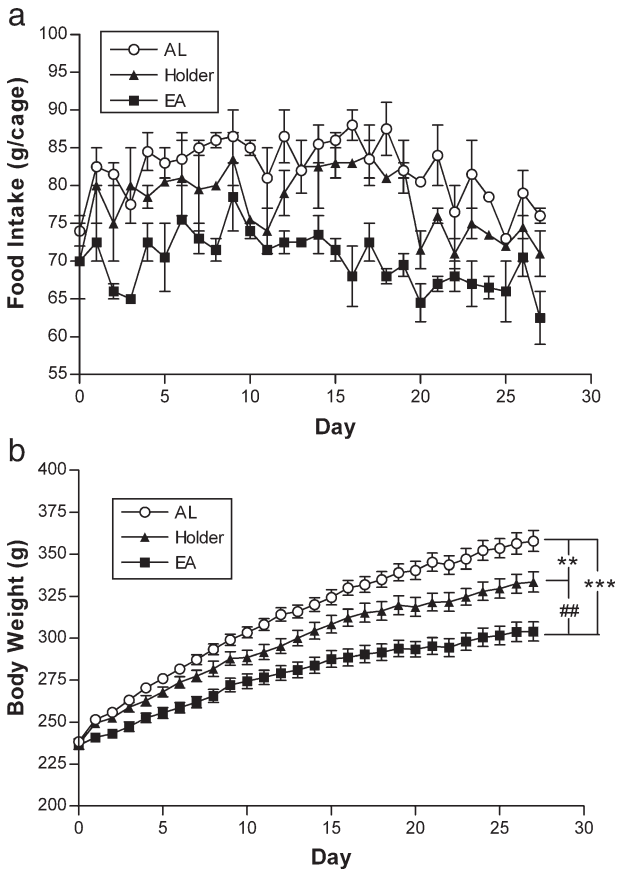

Fig. 2. Changes of daily food intake (a) and body weight (b) in AL, Holder and EA groups. Data are presented as mean±SEM. $N=2$ cages/group (a); $n=8$ rats/group (b). **$p<0.01$, ***$p<0.001$, AL vs Holder or EA; ##$p<0.01$, Holder vs EA by Newman–Keuls multiple comparison test after one-way ANOVA.

3.3. Effects of EA on plasma Epi and NE, and serum corticosterone

The effects of EA on plasma Epi and NE, and serum corticosterone levels are shown in Fig. 4. The plasma Epi levels were significantly increased in the Holder group ($p<0.01$, AL group vs Holder group), but such an increase was not observed in the EA group ($p<0.01$, Holder group vs EA group). There

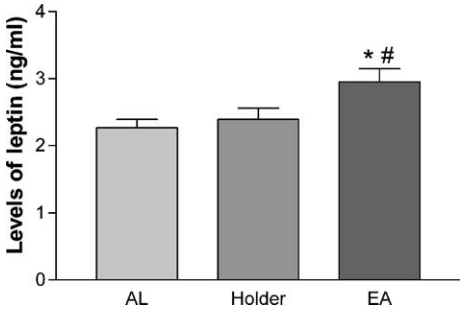

Fig. 3. Effects of EA on serum leptin levels in rats. Data are presented as mean±SEM. $N=8$ rats/group. *$p<0.05$, AL vs EA; #$p<0.05$, Holder vs EA by Newman–Keuls multiple comparison test after one-way ANOVA.

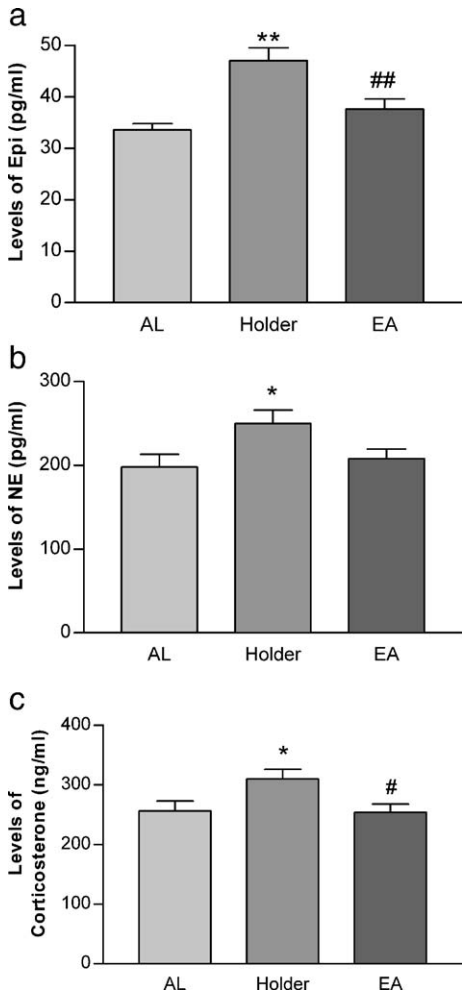

Fig. 4. Effects of EA on plasma Epi (a) and NE (b), serum corticosterone (c) levels in rats. Data are presented as mean ± SEM. N = 8 rats/group. *$p<0.05$, **$p<0.01$, AL vs Holder; $^{\#}p<0.05$, $^{\#\#}p<0.01$, Holder vs EA by Newman–Keuls multiple comparison test after one-way ANOVA.

were no significant differences in Epi levels between AL and EA group ($p>0.05$) (Fig. 4a). The NE levels were also significantly increased in the Holder group ($p<0.05$, AL group vs Holder group), but not in the EA group ($p>0.05$, AL or Holder group vs EA group) (Fig. 4b). Similarly, serum corticosterone levels were significantly increased in the Holder group ($p<0.05$, AL group vs Holder group), but not in the EA group ($p<0.05$, Holder group vs EA group; $p>0.05$, AL group vs EA group) (Fig. 4c).

4. Discussion

4.1. Involvement of leptin in the EA effects on food intake and body weight

Several lines of evidence have indicated that acupuncture or EA treatment effectively reduced food intake and body weight in rats and humans (Gadzhiev et al., 1993; Shiraishi et al., 2003; Lacey et al., 2003; Tian et al., 2005). For example, Gadzhiev et al. (1993) showed that EA treatment reduced body mass and fatty tissue content, and normalized lipid metabolism in obese children. Shiraishi et al. (2003) reported that auricular acupuncture stimulation reduced body weight by enhancing lipid metabolism in both non-obese and mildly obese humans. In obese rats, food intake and body weight were decreased by both the low and high frequency EA treatments (Tian et al., 2003, 2005). The present results also revealed that EA reduces food intake and body weight in normal rats. As a result, it appears likely that EA treatment is indeed effective in regulating food intake and body weight in both normal and obese subjects.

There are some reports showing that acupuncture or EA could affect the hypothalamic neurons with regard to the control of food intake and body weight. In normal SD rats, pro-opiomelanocortin (POMC) mRNA expression in the brain was enhanced as a result of EA treatment (Zhu et al., 1997). In normal or experimentally induced obese rats, acupuncture was able to increase the excitability of the VMH (Asamoto and Takeshige, 1992; Shiraishi et al., 1995; Zhao et al., 2000). It was also reported that acupuncture decreases NPY expression in the arcuate nucleus of the hypothalamus of rats with streptozotocin-induced diabetes, suggesting that acupuncture treatment may be effective in curbing the hyperphagia of diabetes (Lee et al., 2004). Recently, Tian et al. (2003, 2005) showed that low and high frequency EA up-regulated α-MSH and CART expression in the arcuate nucleus of the hypothalamus of rats with high fat diet-induced obesity. Similarly, it is well established that the increased leptin levels in the bloodstream reduce food intake and increase energy expenditure by activating the α-MSH/CART neurons and inhibiting the NPY/AgRP neurons in the arcuate nucleus of the hypothalamus, resulting in the reduction of body weight (Friedman, 2000; Bear et al., 2001; Ahima and Osei, 2004). In the present study, we thus hypothesized that EA might reduce food intake and body weight by increasing the plasma leptin levels. As we predicted, the present results show that EA significantly increase the serum leptin levels when compared with the AL or Holder group of rats. Therefore, we propose that EA reduce food intake and body weight by activating the expression of anoretic peptides and inhibiting the expression of orexigenic peptides in the hypothalamus via the increase of leptin levels.

4.2. Stress and EA effects

Several studies have demonstrated that chronic exposure to stressors may alter food intake and body weight of rats (Marti et al., 1994; Harris et al., 1998; Gamaro et al., 2003). It has been reported that immobilization stress may induce anorexic effects (Marti et al., 1994; Faraday, 2002; Tian et al., 2005). As holder restraint during EA treatment is all but inevitable under our experimental design, it is therefore possible that immobilization stress caused by the holder restraint, not the effect of EA itself, might be responsible for the reduction in food intake and body weight. In fact, we cannot completely rule out the involvement of immobilization stress since food intake and body weight in the Holder group were also reduced when compared with the AL group (Table 1, Fig. 2). However, the effect of EA was much more profound than that shown in the Holder group. The level of plasma Epi and NE, and serum corticosterone, which are

closely related to stress (Han et al., 1999; Yang et al., 2002), were increased in the Holder group, indicating that animals were indeed experiencing stress under the condition. However, it is noteworthy that the elevation of Epi, NE and corticoserone levels was not observed in the EA group. Consequently, these results suggest that EA may inhibit the restraint-induced stress response in rats rather than enhance it. Previous studies have already reported consistent results that low or high frequency EA suppressed the stress responses caused by tooth pulp stimulation or immobilization (Han et al., 1999; Yang et al., 2002; Tian et al., 2005). In addition, the intensity of EA stimulation used in the present study was quite moderate (0.2∼0.3 mA) when compared with other studies (0.5∼1.5 mA), not making rats squawk during EA stimulation. Therefore, the mechanism of EA effects in reducing food intake and body weight cannot be attributed to stress.

4.3. Conclusions and future perspectives

The results from the present study show that EA markedly reduces food intake and body weight in rats and increases serum leptin levels. In addition, EA suppressed plasma Epi and NE, and serum corticosterone levels. These results suggest that the elevation of serum leptin levels induced by EA treatment may contribute to the reduction in food intake and body weight in rats, and this EA effect does not seem to be due to stress. Therefore, EA treatment may serve as an effective complementary and alternative therapy for the management of food intake and body weight, and this area thus deserves more clinical studies.

At present, the mechanism underlying the enhancing effects of EA on serum leptin levels is unknown. Some factors, such as insulin, glucocorticoids and proinflammatory cytokines (e.g. TNF-α, IL-1), are known to stimulate the secretion of leptin from the adipose tissue (Ahima and Osei, 2004). Chang et al. (1999) reported that EA treatments in diabetic and normal rats increased the plasma insulin concentrations, while serum corticosterone levels in rats (Fig. 4c; Han et al., 1999) and proinflammatory cytokines in humans (Jeong et al., 2003) were reduced by EA. Therefore, it is plausible that insulin could act as an important factor in mediating the enhancing effects of EA on leptin levels. In future studies, the relationship among EA, insulin and leptin will be investigated.

Since young rats were used at the beginning of the experiment, daily EA stimulation for 4 weeks may have affected the normal growth of the rats, contributing to the present results. However, there were no significant differences in serum levels of growth hormone and nose–anus length among the AL, Holder and EA groups (data not shown). It is thus unlikely that the actions of EA in the present study are side effects.

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