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Neuroscience Letters 383 (2005) 17–21
Neuroscience Letters
www.elsevier.com/locate/neulet

Up-regulation of the expression of cocaine and amphetamine-regulated transcript peptide by electroacupuncture in the arcuate nucleus of diet-induced obese rats

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Received 3 February 2005; received in revised form 19 March 2005; accepted 23 March 2005

Abstract

It was reported that acupuncture or electro-acupuncture (EA) is effective in reducing the body weight for obese patients, although the mechanisms remain obscure. In a previous study, we have found that rats fed with high-fat (HIF) diet developed diet-induced obesity (DIO) with a concomitant decrease in the hypothalamic content of the cocaine and amphetamine-regulated transcript (CART) peptide, a peptide with anorexiogenic effect. To assess the central effect of EA on DIO rat, we revealed that EA up-regulated the expression of CART peptide in the arcuate nucleus (ARC) of the DIO rats. After feeding with HIF diet for 14 weeks, the DIO rats received EA stimulation three times per week for 4 weeks. The expression of CART peptide in ARC was measured using immunohistochemistry. The plasma ACTH was measured with ELISA. EA caused a reduction of both body weight and energy intake in DIO rats and increased the expression of CART peptide in ARC. The plasma ACTH was increased in response to restraint stress, but EA produced no further increase in ACTH levels. The results suggest that EA can up-regulate the expression of CART peptide to approach normal level, resulting in an inhibition of food intake and a reduction of body weight in DIO rats.
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Keywords: Electroacupuncture; CART; Body weight; Diet-induced obesity; Arcuate nucleus

In humans and animals, a chronic high-fat (HIF) diet without a compensatory increase in energy expenditure leads to the progressive development of obesity [12]. In the majority of obese patients, dietary therapy is the first line of treatment. However, many patients achieve only minor weight loss and have difficulty in maintaining the weight loss with dietary treatment alone [24]. Acupuncture has been widely used in China for thousands of years. It has taken many years for the western medical establishment to acknowledge the effectiveness of acupuncture, and even today, it is not generally regarded as scientifically acceptable. The problem has always been the difficulty to explain the mechanisms behind the effects of acupuncture, and to demonstrate its effects in controlled clinical trials [9].

As in much of human obese cases, the rat model of diet-induced obesity (DIO) appears to follow a polygenic mode in inheritance. Hypothalamic neuropeptides play an important role in regulating energy balance [13]. A dysregulation in the hypothalamic neuropeptides has previously been shown in models of DIO [10,19]. Cocaine and amphetamine-regulated transcript (CART) peptide is one of the most abundant hypothalamus specific transcripts, distributed in hypothalamic nuclei, including arcuate nucleus (ARC) [7]. This peptide has been shown to inhibit feeding when administered centrally

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doi:10.1016/j.neulet.2005.03.039

[22]. Our previous studies have shown that there is a down-regulated expression of CART and α-melanocyte-stimulating hormone (α-MSH) in DIO rats [18], and that electroacupuncture (EA) can up-regulate the expression of α-MSH in the hypothalamic ARC of the DIO rats [16]. The aim of the present study was to evaluate whether EA can modulate the expression of the CART peptide in hypothalamic ARC of the DIO rats.

One hundred male Sprague–Dawley rats (45–55 g) were randomly divided into two groups: (1) the control group ($n = 20$), fed with standard laboratory chow (15.91 kJ/g); (2) the HIF diet group ($n = 80$), fed with HIF diet consisting of 30% fat, 40% carbohydrate, 15.5% protein, containing 19.93 kJ/g. The animals were housed in air-conditioned rooms ($22 \pm 2\,^{\circ}C$) with lights on between 07:00 and 19:00 h and with free access to food and water. Body weight was monitored once every week. All procedures were performed in accordance with institutional guidelines of the Animal Care Committee of the Peking University. After feeding for 14 weeks, rats in the HIF diet-feeding group with higher body weights were assigned as DIO rats ($n = 40$, 50%), their body weights surpassed the maximum body weights of rats in the control group. DIO rats were divided into the following four groups ($n = 10$ for each group): 2 Hz EA group, 100 Hz EA group, the restraint group and the DIO group receiving no further treatment serving as control. Rats of each group were housed individually and acclimated to the new environment for one week.

Rats of 2 Hz EA, 100 Hz EA and the restraint group were kept in restrainers during the EA treatment. Stainless steel acupuncture needle was 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, or 0.2 ms at 100 Hz); intensities ranging from 0.5, 1.0 to 1.5 mA, with each intensity lasting for 10 min. The frequency was set at 2 Hz or 100 Hz. EA experiment was performed in 14:00–15:00 h. EA was given 30 min per session, three times per week for 4 weeks. Rats in the restraint control group were restrained for 30 min without EA. In all groups, food intake and body weight were measured daily.

Rats were anaesthetized with sodium pentobarbital (50 mg/kg, i.p.) immediately after the final EA treatment. Cardiac blood (2 ml) was collected into heparinized tubes, and centrifuged at 12,500 rpm, 4 °C for 5 min. ELISA was used to measure plasma levels of adrenocorticotropic hormone (ACTH) with commercial kits (Phoenix, CA) according to the manufacturer's instructions.

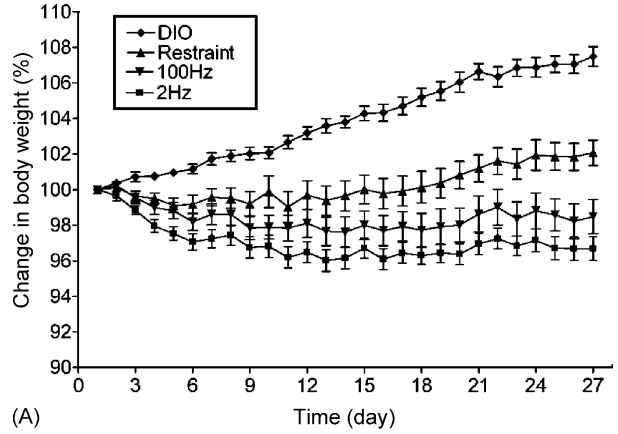
After blood collection, rats were transcardially perfused with isotonic saline followed by 4% paraformaldehyde and the brains were removed. The brains were frozen at −70 °C. Serial coronal sections of 40 μm thickness were prepared at ARC level. The sections were pre-incubated in a solution containing 4% normal goat serum, 1% bovine serum albumin and 0.3% Triton X-100. After washing with PBS, the sections were incubated with rabbit anti-CART peptide antiserum (Phoenix, CA) at a dilution of 1:4000, for 48 h at 4 °C. The sections were then incubated with anti-rabbit biotinylated IgG (1:200, Vector) for 4 h, followed by incubation with streptavidin–biotin–peroxidase complex (1:200, Vector) for 2 h at room temperature. They were then developed in 0.1 M acetate buffer containing 0.02% diaminobenzidine, 4% nickel ammonium sulphate and 0.03% H_2O_2 for 10 min. The sections were mounted onto gelatin-coated slides, dehydrated through graded series ethanol solutions followed by xylene.

The CART 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 CART 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 ARC. All data were analyzed using one-way ANOVA followed by the Newman–Keuls comparison test. $P < 0.05$ was considered statistically significant.

Before EA treatment, the average body weight of DIO rats (729.9 ± 8.2 g) was 25.6% greater than that of the control group (580.9 ± 13.0 g) ($P < 0.001$). After four week EA treatment, there was a significant decrease of the average body weight of the rats in 2 Hz (−3.32%) and 100 Hz EA group (−1.52%). At the same time, the average body weight of the rats in the restraint group and the DIO group were increased for 2.1% and 7.5%, respectively. The differences in changes of body weight between the EA treatment groups and the restraint or DIO group were very significant ($P < 0.001$) (Fig. 1).

During the same period, a significantly higher energy intake per day was observed in the DIO group (584.7 ± 11.1 kJ/day) as compared to the control group (468.5 ± 22.0 kJ/day) ($P < 0.001$). The energy intake of the EA groups was significantly lower in 2 Hz (430.1 ± 10.7 kJ/day) and 100 Hz group (432.0 ± 9.4 kJ/day) as compared to the DIO group ($P < 0.001$). Energy intake of the restraint group was significantly lower than that of the DIO group, but is still significantly higher than the two EA groups ($P < 0.001$) (Fig. 2).

The 2 Hz EA group and the restraint group had significantly higher levels of ACTH in the plasma than the control group and the DIO group ($P < 0.05$). In contrast to that of the 2 Hz EA group, plasma level of ACTH in 100 Hz EA group showed a slight decrease compared with the restraint group, although the difference between these two groups was not statistically significant (Fig. 3).



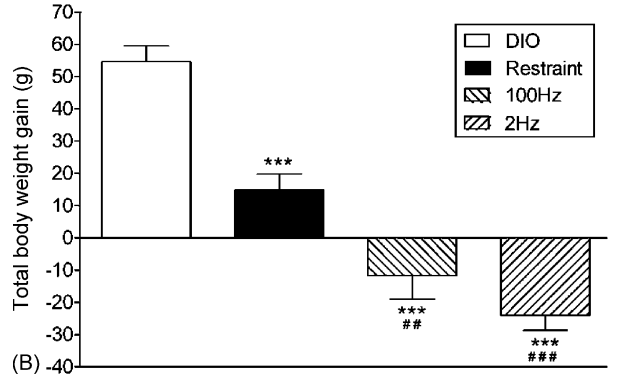


Fig. 1. (A) Percent change in body weight of the rats during EA treatment for 4 weeks. The body weight of the rat assessed immediately prior to EA treatment was taken as 100%. (B) Total body weight gain (g) of the rats after EA treatment. Body weight assessed immediately prior to EA treatment was taken as the reference. Data are given as the mean ± S.E.M. (bar). $^{***}P < 0.001$ vs. DIO group; $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. restraint group.

Typical distribution of CART peptide-immunoreactive neurons in the hypothalamic ARC in the rat brain is shown in Fig. 4. A significant reduction of CART peptide-immunopositive cells was observed in the DIO group com-

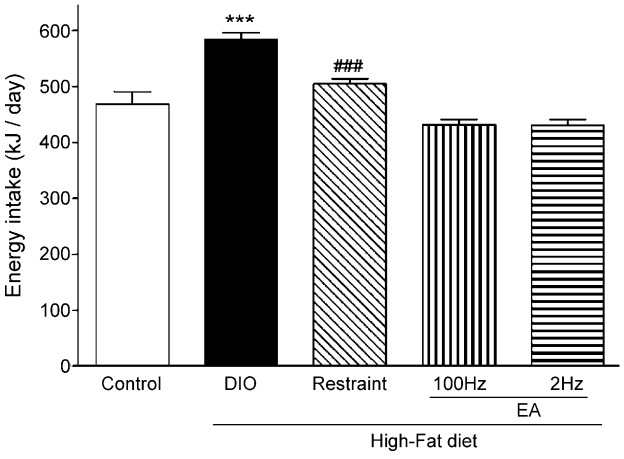


Fig. 2. Energy intake (kJ/day) of rats fed with an HIF diet and the control group fed with standard chow. EA was administered for 4 weeks. Data are given as the mean ± S.E.M. (bar). $^{***}P < 0.001$ vs. each of other groups; $^{\#\#\#}P < 0.001$ vs. 2 Hz and 100 Hz group.

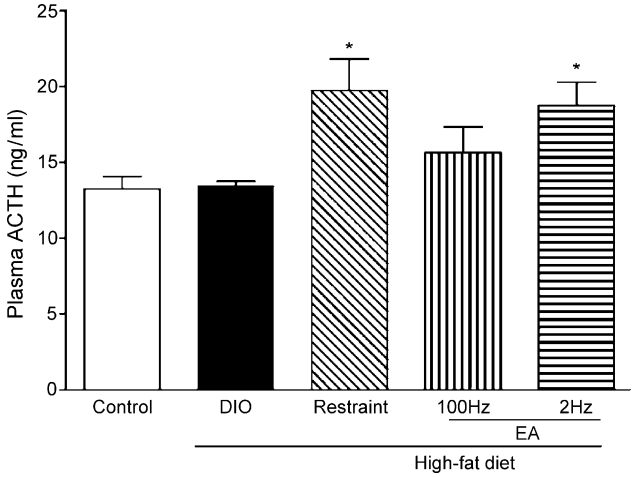


Fig. 3. Plasma level of ACTH in rats fed with a high-fat diet and the control group fed with standard chow. EA was administered for 4 weeks. Data are given as the mean ± S.E.M. (bar). $^{**}P < 0.01$ vs. control and DIO group.

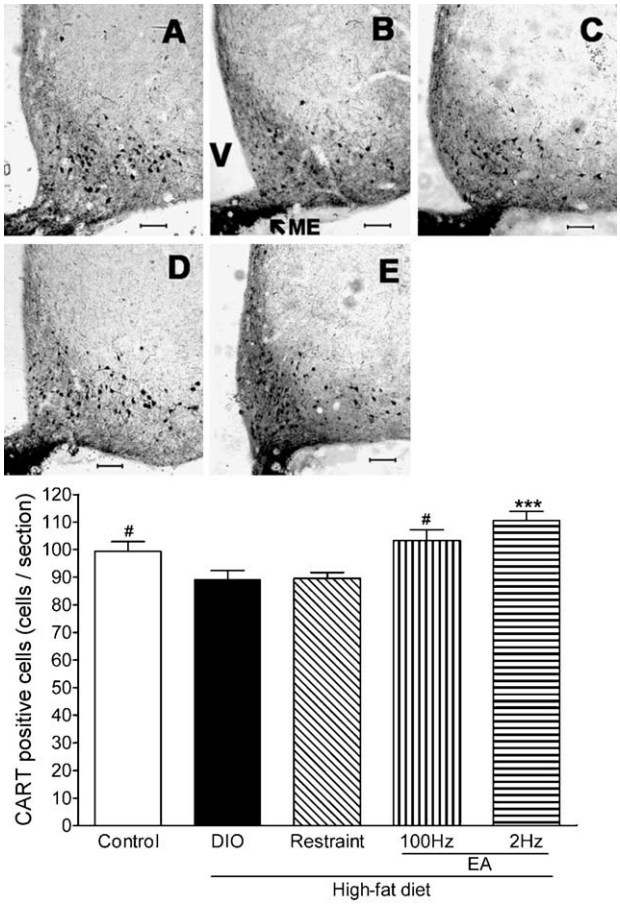


Fig. 4. Upper panel: photomicrograph illustrating the distribution of CART peptide-immunoreactive neurons in hypothalamic ARC of control rats (A), DIO rats (B), restraint rats (C), 100 Hz EA rats (D), and 2 Hz EA rats (E). V: third ventricle; ME: median eminence. Scale bar represents 100 μM. Lower panel: number of CART peptide-immunoreactive positive cell in ARC of hypothalamus in rats fed with a high-fat diet, and the control group fed with standard chow. EA was administered for 4 weeks. Data are given as the mean ± S.E.M. (bar). $^{***}P < 0.001$ vs. DIO and restraint group; $^{\#}P < 0.05$ vs. DIO and restraint group.

pared with the control group. This reduction could be reversed by the 2 Hz EA or the 100 Hz EA, but not by the simple restraint procedure (Fig. 4).

In the previous studies, we have shown that feeding HIF diet to the rat caused an increase of food intake and body weight (DIO rats), accompanied by a down regulation of the expression of CART and α-MSH in the ARC of hypothalamus [18]. Down regulation of the expression of α-MSH in the ARC of hypothalamus in DIO rats could be effectively reversed by EA stimulation [16]. The purpose of the present study was to observe whether the down-regulated expression of the hypothalamic CART peptide in DIO rats can also be reversed by EA stimulation. Results in Fig. 4 clearly indicate an increase in the expression of CART peptide in hypothalamic ARC in rats subjected to EA stimulation to approach the normal level. The results are in favor of, although not an approval of, the hypothesis that an increase of the CART expression in the hypothalamus may play a role in mediating the anti-obesity effect of EA.

The administration of EA requires partial immobilization of the rat in a restrainer, which may cause certain degree of stress to the animal. Since immobilization stress of sufficient intensity may induce anorexic effects [3], the restraint of the animal during EA may represent a major confounding variable for the assessment of the efficiency of EA in the control of food intake and body weight. Thus, we set a group of simple restraint without EA to control the factor of immobilization stress, using plasma ACTH as a representative marker for stress hormones [5]. The results show that simple restraint does increase plasma ACTH level for 46.7% ($P < 0.05$, compare with the DIO group), yet the 2 Hz EA group produces no more elevation of ACTH level. This result suggests that the mild EA treatment used in the present study seems not to enhance the restraint-induced stress response. In fact, in the 100 Hz EA group, plasma ACTH level was even slightly lower than that of the simple restraint group, suggesting that 100 Hz EA may serve as a therapeutic measure against stress response [5]. Results obtained in the present study indicate that while acupuncture inevitably contains a component of stress, the mechanism of action of EA in suppressing food intake in DIO rats can not be attributed to the factor of stress.

Rodent model of DIO has been useful in understanding the role of the brain in regulating energy homeostasis [19]. It is well known that ARC of hypothalamus plays an important role in energy balance [21]. We, therefore, focus in the present study on the hypothalamic CART peptide that has been found in brain areas involved in physiological control of feeding behavior [7]. Central injection of CART peptide is effective in inhibiting feeding behavior, favoring lipid oxidation and limiting fat storage both in normal and DIO rats [8,11]. CART-deficient mice are significantly heavier when fed an HIF diet [2]. These data constitute strong evidence of a role for endogenous CART peptide in the regulation of feeding behavior and body weight.

Alternative medicine is a part of the physician's therapeutic armamentarium [20] and acupuncture seems to have acquired a leading position among alternative therapeutic methods [4]. Acupuncture is among the most popular of all complementary and alternative therapies in the medical management of obesity [9,14,17,23]. Studies on the mechanisms of acupuncture treatment for obesity are emerging increasingly with time. Shiraishi et al. reported that acupuncture clearly modulates feeding-related hypothalamic neuronal activity of obese rats [15]. Asamoto and Takeshige showed that stimulation of the rat inner auricular regions produced evoked potentials in the hypothalamic ventromedial nucleus and reduced body weight of the rats [1]. Auricular acupuncture was reported to decrease neuropeptide Y (NPY) expression in the hypothalamus of food-deprived rats [6]. No study has so far been revealed to relate hypothalamic CART peptide with acupuncture effect on the control of DIO.

In conclusion, EA can up-regulate CART peptide in hypothalamic ARC to approach normal level, which may play a role in reducing food intake and body weight in DIO rats. While partial immobilization for EA treatment may induce certain degree of stress, this should not be regarded as an important factor responsible for the anorexiogenic effect of acupuncture.

Acknowledgements

This research was funded by the National Natural Science Foundation of China (30270624) to D.-R. Tian, and a grant from the Ministry of Science and Technology (973 project 2003CB515407) to J.-S. Han.

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