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Changes of hypothalamic α-MSH and CART peptide expression in diet-induced obese rats

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Abstract

Two hypothalamic peptides, cocaine and amphetamine-regulated transcript (CART) and α-melanocyte-stimulating hormone (α-MSH), recognized as anorexigenic neuropeptides to suppress the feeding behavior, were monitored in rats fed with a high-fat (HIF) diet for 14 weeks. While half of the rats developed obesity (diet-induced obese, DIO), some did not (diet resistant, DR). Compared to the DR rats and the control rats (fed with standard chow), DIO rats were accompanied by a markedly higher energy intake and a decrease in the number of neurons carrying α-MSH and CART peptide in the arcuate nucleus of the hypothalamus. Failure of hypothalamic anorexigenic peptides CART and α-MSH to increase their content in response to HIF diet may play a key role for overly high energy consumption, resulting in obesity.

Keywords: Cocaine and amphetamine-regulated transcript peptide; α-Melanocyte-stimulating hormone; Diet-induced obesity; High-fat diet; Hypothalamus

1. Introduction

Feeding behavior and body weight are controlled through complex interactions between the central nervous system and peripheral organs. Food intake, especially the meal size, is controlled by a series of short-term hormonal and neural signals which is derived from the gastrointestinal tract, such as cholecystokinin [3] and ghrelin [9,10,26]; while long-term energy stores are signaled by other hormones such as insulin and leptin [16]. These signals seem to modulate the expression of orexigenic and anorexigenic neuropeptides in the hypothalamus and other brain regions, culminating in changes in food intake and energy expenditure for the control of energy homeostasis [14,31]. In recent years, much evidence has accumulated on a number of hypothalamic neuropeptides, including neuropeptide Y (NPY), agouti-related protein, cocaine and amphetamine-regulated transcript (CART) peptide, and proopiomelanocortin (POMC)-derived peptides such as α-melanocyte-stimulating hormone (α-MSH) [2]. Both CART peptide and α-MSH have been shown to inhibit feeding behavior when administered centrally. In addition, they increase sympathetic outflow and thermogenic activity resulting in an increased energy expenditure [7,13,25,28].

In animals and humans, a chronic high-fat (HIF) diet without a compensatory increase in energy expenditure leads to the progressive development of obesity [11,29]. The model of diet-induced obese (DIO) animal has been proven useful as a model for human obesity [18]. As in much of human obese cases, the animal model of DIO appears to follow a polygenic mode in inheritance. Thus, the physiological changes observed in this animal model should provide a useful insight into the development of obesity in humans. Several previous experiments have dealt with diet-induced changes

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in hypothalamic gene expression of neuropeptides in rodents. Thus, CART mRNA is heavily expressed in the hypothalamus [6], which is reduced by fasting, and also by genetic leptin deficiency and leptin resistance [17,22]. Rodents prone to DIO are accompanied by an elevated level of NPY mRNA and decreased POMC mRNA in the arcuate nucleus (ARC). On the other hand, decreased NPY mRNA and increased POMC mRNA were found in diet resistant (DR) animals [4,19,21,23,35].

To gain further insight into the physiological response of the rat to an HIF diet, we investigated the food intake, energy intake, body weight and the expression of CART peptide and α-MSH in the hypothalamic arcuate nucleus (ARC) of the rats when they were chronically exposed to HIF diet. Emphasis was put on the possible difference in peptide expression between rats that did or did not develop obesity with the same HIF diet (DIO versus DR).

2. Research methods and procedures

2.1. Establishment of DIO model in rats

Three-week-old male (45–55 g) Sprague–Dawley (SD) rats were obtained from Vital Company, Beijing. Animals were housed in a facility with controlled temperature (22 ± 2 °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 guidelines of the Animal Care Committee of the Peking University.

Animals were then randomly divided into two groups: (1) the control group (n = 20), fed with standard laboratory chow (Vital Company Beijing), consisting of 5% fat, 55% carbohydrates, 22% protein, 7% ash and 5% fiber (3.80 kcal/g); (2) the HIF diet group (n = 80), fed with HIF diet consisting of 50% standard laboratory chow, 15% lard, 10% sucrose, 5% powdered milk, 10% egg, 5% sesame oil and 5% peanut kernel. The HIF diet consisted of 30% fat, 40% carbohydrate, 15.5% protein, 4% ash, and 3% fiber, containing 4.76 kcal/g. Body weight was monitored once every week.

After feeding for 14 weeks, rats in HIF diet-feeding group, showing higher body weights, were assigned to the DIO group (n = 40, 50%), their body weights surpassed the maximum body weights of rats in the control group. Rats of the HIF diet group with lower body weight were assigned to the DR group (n = 13, 16%), their body weights were less than the average of the control group. In the HIF diet group, the rats with body weight falling into the range between the average and the maximum body weights of the control group (n = 27, 34%) were discarded. Ten rats of DIO and DR group respectively, and 20 rats of control group were housed individually, and fed with the corresponding diet. Food intake and body weight were measured daily, for 4 weeks.

2.2. Immunohistochemical procedure

Rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and were transcardially perfused with 150 ml of isotonic saline followed by 250 ml of 4% paraformaldehyde. After perfusion, the brains were removed from the skulls and placed in the same fixative overnight at 4 °C. After post-fixation, the brains were kept sequentially in 10, 20 and 30% sucrose solution respectively for 24 h each at 4 °C for cryoprotection, and were then frozen at −70 °C. Serial coronal sections of 40 μm thickness were prepared at ARC level with Cryostat (Leica, Nussloch, Germany). A consistent angle of cut was maintained by examining the shape of the third ventricle and anterior commissure. To ensure reliable comparisons among different groups and to maintain stringent tissue preparation and staining conditions, all the brains from different groups were processed at the same time.

Free-floating staining method was used. Tissue sections were pre-incubated in a solution containing 4% normal goat serum, 1% bovine serum albumin and 0.3% Triton X-100 at 37 °C for 30 min. After washing with PBS, sections were incubated with primary antibodies (rabbit–anti-rat antiserum against α-MSH or CART peptide at a dilution of 1:4000, Phoenix, CA) for 48 h at 4 °C. The sections were then incubated with anti-rabbit biotinylated IgG (1:200, Vector Labs, Burlingame, CA) for 4 h at room temperature. The sections were washed in PBS, and then incubated with the streptavidin–biotin–peroxidase complex (1:200, Vector Labs) for 2 h at room temperature. The sections were then developed in 100 mmol/L acetate buffer containing 0.02% 3,3′-diaminobenzidine (DAB, Sigma), 4% nickel ammonium sulphate and 0.03% H_2O_2 for 10 min at room temperature. All of the incubations and washing steps were carried out on an orbital shaker. After the immunohistochemical procedure, the sections were mounted onto gelatin-coated slides, dehydrated through graded series ethanol solutions followed by xylene.

After coverslip, the sections were examined under an Olympus light microscope. All the α-MSH and CART peptide-immunoreactive neurons were counted under 10× magnification according to the atlas of the rat brain in stereotaxic coordinates by Paxinos and Watson. Counting was done in a blind manner as to the identity of the sample by a single observer. Only cell bodies that clearly exhibited cytoplasmic staining were scored as positive. Six sections were counted for α-MSH or 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 hypothalamic ARC.

2.3. Statistical analysis

Results were expressed as mean ± S.E.M. Food intake, body weight and the number of the immunoreactive neurons of the DIO, DR and control group were analyzed

using one-way analysis of variance (ANOVA), followed by Newman–Keuls comparison test. Differences were considered statistically significant for $P < 0.05$.

3. Results

3.1. Effect of HIF diet on body weight and energy consumption in DIO and DR rats

Initial average body weight of the rats in the control group (52.2 ± 1.3 g) did not differ significantly from that in the HIF diet group (52.7 ± 0.6 g) ($P > 0.05$). Six weeks later, difference in body weight became apparent between the two groups ($P < 0.05$). At the fifteenth week, the average body weight of the control group was 551.7 ± 10.9 g, compared with 635.3 ± 11.6 g in HIF diet group, which was 14.6% greater than that of the control group ($P < 0.01$) (Fig. 1). In the HIF diet group, the average body weight of DIO rats was 698.9 ± 6.7 g ($n = 40$), as compared to 521.5 ± 6.6 g ($n = 13$) in DR rats ($P < 0.01$). Body weight of DIO rats was 34.0% greater than that of the DR rats ($P < 0.01$) (Fig. 2).

During the period of 16–19 week, a significantly higher energy intake per day was observed in DIO group (144.4 ± 2.9 kcal/day) as compared to the DR (119.7 ± 2.4 kcal/day) and the control group (108.0 ± 4.9 kcal/day) ($P < 0.001$) (Fig. 3A). The amount of food intake in the DIO group (29.1 ± 0.58 g/day) remained at the same level as the control group fed with the standard chow (29.0 ± 1.28 g/day), yet that of the DR group was significantly less than the DIO group (23.6 ± 0.49 g/day) (−17%, $P < 0.001$) (Fig. 3B).

3.2. CART peptide expression in ARC

Typical distributions of CART peptide-immunoreactive neurons in the hypothalamic ARC in the rat brain were shown in Fig. 4. A large number of CART peptide-immunoreactive neuronal perikarya and fibers were observed in the ARC. Compared to DR rats (105.7 ± 3.5 cell/section) and control rats (99.3 ± 4.4 cell/section), a significant reduction in the number of CART peptide neurons was observed in the DIO rats (89.3 ± 2.1 cell/section) ($P < 0.01$) (Fig. 5), showing a decrease of 15.5 and 10.1%, respectively.

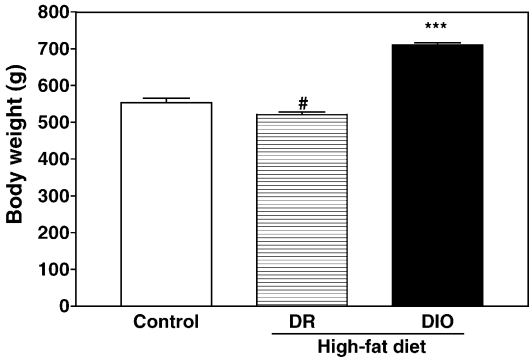

Fig. 1. The body weight of diet-induced obese (DIO, $n = 40$), diet resistant (DR, $n = 13$) and control rats ($n = 20$) after respectively feeding a high-fat diet (4.76 kcal/g) or standard chow (3.80 kcal/g), respectively for 14 weeks. Data are mean ± S.E.M. $^{***}P < 0.001$ vs. control and DR group; $^{\#}P < 0.05$ vs. control group.

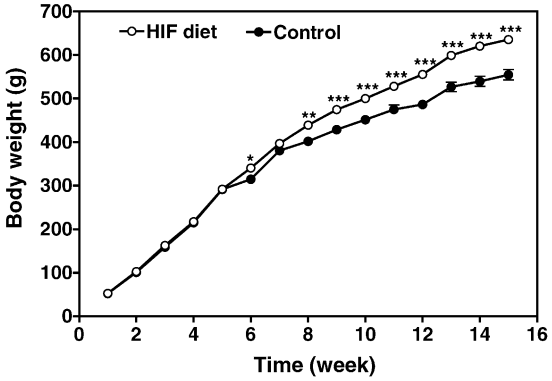

Fig. 2. The body weight of SD rats fed respectively with a high-fat (HIF) diet (◯) (4.76 kcal/g, $n = 80$) and standard chow (□) (3.80 kcal/g, $n = 20$) for 14 weeks, respectively. At the first week, both groups were fed with standard chow. Each point represents the mean ± S.E.M. of the groups. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ vs. control group.

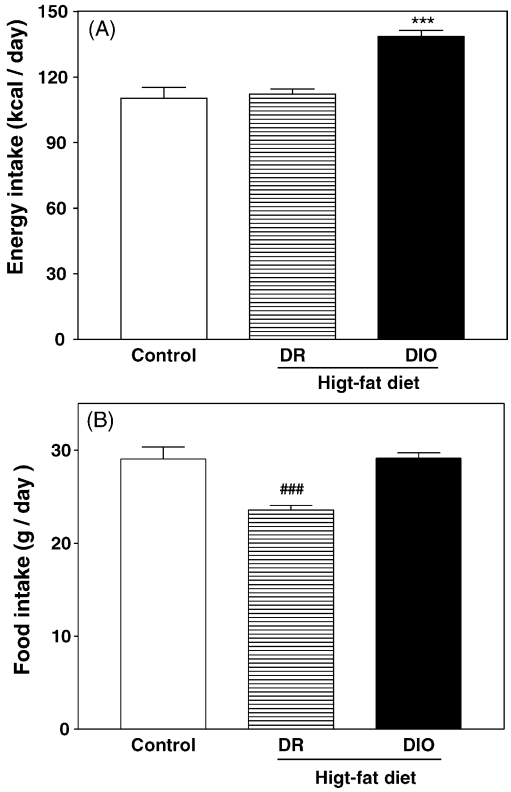

Fig. 3. Energy intake (kcal/day) (A) and Food intake (g/day) (B) of rats fed with a high-fat diet (4.76 kcal/g). The control group was fed with standard chow (3.80 kcal/g). Each bar represents the mean ± S.E.M. of the 10 animals. $^{***}P < 0.001$ vs. control and DR group; $^{\#\#\#}P < 0.001$ vs. control and DIO group.

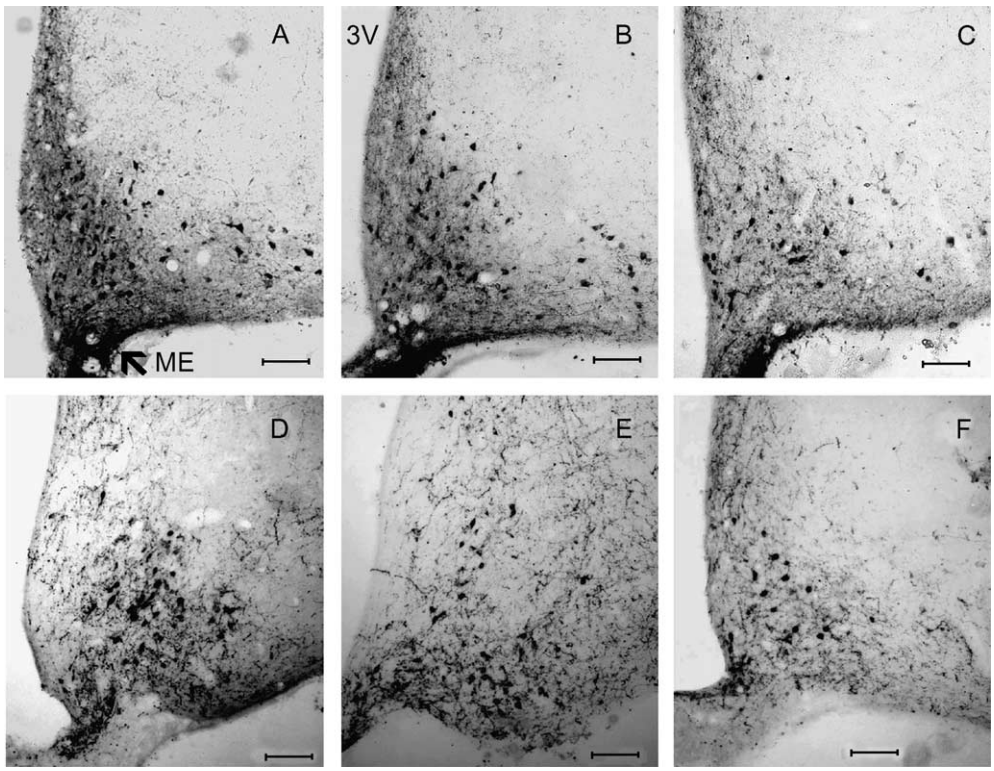

Fig. 4. Photomicrographs illustrating the distribution of CART peptide- and α-MSH- immunoreactive neurons in hypothalamic ARC. Control rats/CART peptide (A), diet resistant rats/CART peptide (B), diet-induced obesity rats/CART peptide (C), Control rats/α-MSH (D), diet resistant rats/α-MSH (E), diet-induced obesity rats/α-MSH (F). 3V: third ventricle; ME: median eminence. Scale bar represents 100 μm.

3.3. α-*MSH expression in ARC*

In the ARC of hypothalamus, the number of α-MSH-immunoreactive neurons was less than that of CART-immunoreactive neurons (Fig. 4). Compared to DR rats (51.4 ± 1.0 cell/section) and control rats (57.6 ± 2.5 cell/section), a significant reduction in the number of α-MSH neurons was observed in the ARC of the DIO rats (37.6 ± 1.5 cell/section) ($P < 0.001$) (Fig. 6), showing a decrease of 26.8 and 34.79%, respectively.

4. Discussion

Obesity, one of the most prevalent diseases in the developed countries, is associated with the consumption of a

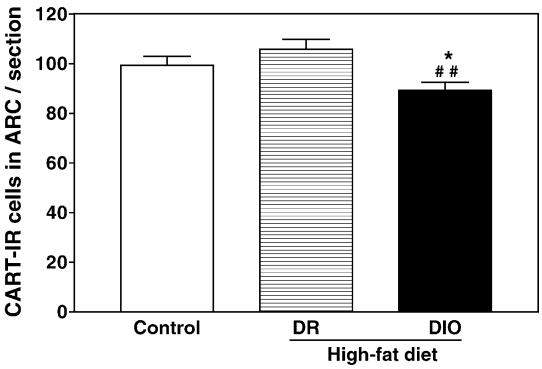

Fig. 5. Number of CART peptide immunoreactivity positive cell in ARC of hypothalamus in rats fed with a high-energy diet (4.76 kcal/g). The control group was fed with standard chow. Each bar represents the mean ± S.E.M. of the five animals (6 section/animal). $^{*}P < 0.05$ vs. control group; $^{\#\#}P < 0.01$ vs. DR group.

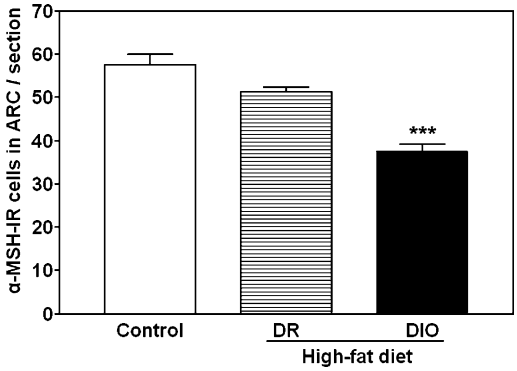

Fig. 6. Number of α-MSH immunoreactivity positive cell in ARC of hypothalamus in rats fed with a high-energy diet (4.76 kcal/g). The control group was fed with standard chow (3.80 kcal/g). Each bar represents the mean ± S.E.M. of the five animals (6 section/animal). $^{***}P < 0.001$ vs. control and DR group.

high-energy diet [30]. A subset of the population appears to be sensitive to diet-induced obesity, while others can maintain normal body weight on the same diet. The task of identifying causative or contributory factors is made difficult because of the multi-etiologic nature of obesity and the multiple metabolic perturbations that occur in obese individuals. The rat model of DIO has been useful in studying the role of the brain in regulating feeding behavior and energy homeostasis [15,24,30,38]. The metabolic profile of DIO rats bears resemblance to that of obese humans such as insulin resistance, dyslipidemia and hyperleptinemia [20,30,36].

The main purpose of the present study was to observe whether there is a significant difference in the expression of some anorexigenic neuropeptides (CART peptide and α-MSH) in ARC between DIO and DR rats. It was interesting to note that while there was a general trend of increase in energy intake and body weight in response to chronic HIF diet, SD rats fed with the same diet for 14 weeks diverged into two populations. About half of the rats developed DIO with body weight higher than the highest in the control group fed with standard chow, and the rest are within the range of control group. About 16% of the rats were designated as diet resistant (DR) rats according to our stringent criteria (body weight less than the average of control group). It is evident from the dietary record that (a) the consumption of food and energy of the DIO rats was significantly higher than that of the DR rats; (b) the DIO rats took the same amount of food pellets despite the change in diet composition; (c) the DR rats consumed significantly less amount of HIF diet to keep the energy consumption equal to that of the control group. These results clearly show that the development of obesity is accounted for by both environmental factors (e.g., diet composition) and genetic factors (individual variation). To explore the possible mechanisms, we tried to correlate the feeding behavior and body weight changes of the rats with the changes in the expression of hypothalamic neuropeptides. It was especially noticeable that DIO rats were accompanied by a marked down-regulation of α-MSH and CART peptide-positive neurons in ARC of hypothalamus, relative to the DR and control groups. Since α-MSH and CART peptide are known to have a potent effect of decreasing food intake and body weight [25,34], a reduction in the number of α-MSH and CART peptide-positive neurons in ARC of DIO rats may have contributed to the increase in food intake and body weight.

The brain responds to altered energy homeostasis by adjusting feeding behavior and energy expenditure. The hypothalamus has long been recognized as a major site in the central nervous system where a spectrum of internal and external environmental information is integrated for energy homeostasis. Previous studies have examined the physiological regulation exerted by nutritional factors on the hypothalamic neuropeptides involved in energy homeostasis. Thus, acute food deprivation leads to down regulation of POMC mRNA in rodents [5]. Conversely, POMC mRNA expression is up-regulated in overfed rats [12]. Concerning the hypothalamic regulation of POMC mRNA expression in rodents feeding with an HIF diet, Ziotopoulou et al. found a marked increase in POMC mRNA levels, 2 weeks after feeding with an HIF diet in C57Bl/6J mice [39], which are among the more sensitive strains in response to dietary intervention [33,37]. However, Lin et al. demonstrated in the same strain of mice that after 19 weeks of HIF feeding, ARC POMC mRNA levels showed a 55% decrease [23], suggesting that the hypothalamic regulation bears a temporal course of response, i.e. an attempt of compensation in the early stage, and a failure of compensation in the late stage. In other words, when animals are fed with high fat diet chronically, a dysfunction of this compensatory mechanism occurs in some individuals, which leads to obesity.

In contrast to DIO mice, the DR mice showed an increased level of POMC mRNA after 14 weeks on an HIF diet [4]. Since POMC is the precursor of α-MSH, a potent inhibitor of food intake, the hypothalamic over-expression of POMC is a natural feedback mechanism in response to high fat diet, in order to maintain homeostasis of energy balance and body weight.

CART peptide is among the most recently discovered putative peptide neurotransmitter [8,32]. Robson found that the marked decrease in CART mRNA expression in the ARC in gold thioglucose-lesioned mice may contribute to the development of obesity [27]. Central injection of recombinant CART peptide is effective in inhibiting feeding behavior [17], favoring lipid oxidation and the reduction of fat storage both in normal and DIO rats [28]. CART-deficient mice were significantly heavier when fed an HIF fat diet than on a regular chow diet at the 14th week of the feeding studies [1]. Furthermore, CART peptide is found in neurons regulating sympathetic outflow, which in turn play an integral role in regulating body temperature and energy expenditure [7]. Taken together, these data constitute strong evidence of a role for endogenous CART peptides in the regulation of feeding behavior and body weight. To our knowledge, a decrease of CART peptide positive neurons in ARC of DIO rats, shown in the present study, had not been reported before.

It should be noted that if the reduction of food intake of the DR rats is due to a compensatory mechanisms of over expression of CART and/or α-MSH peptide, then there should be an increase of CART/α-MSH positive neurons in hypothalamic ARC compared to normal rats. This was, however, not found in hypothalamic sections. An alternative possibility is that a seemingly unchanged tissue level of the neuropeptide is indeed a result of an accelerated synthesis and an exaggerated release. This uncertainty can only be resolved by the simultaneous assessment of localized peptide release and the level of gene expression.

In conclusion, the effect of long term HIF diet on the feeding behavior of rats seems to have significant individual variation. A small fraction of the rats remained in normal body weight by compensatory reduction of food intake to keep the energy consumption at a constant level. Most of the rats, however, showed an increase of body weight due to an increase

in energy intake. This is accompanied by a reduction in the number of neurons carrying the anorexigenic neuropeptides CART and MSH in the hypothalamus. It is speculated that a dysregulation of the compensatory mechanism of hypothalamic anorexigenic neuropeptides may account for the obesity observed in rats, fed chronically with high fat diet.

Acknowledgments

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References

[1] Asnicar MA, Smith DP, Yang DD, et al. Absence of cocaine- and amphetamine-regulated transcript results in obesity in mice fed a high caloric diet. Endocrinology 2001;142:4394–400.
[2] Beck B. Neuropeptides and obesity. Nutrition 2000;16:916–23.
[3] Beglinger C. Cholecystokinin and eating (overview). Curr Opin Investig Drugs 2002;3:587–8.
[4] Bergen HT, Mizuno T, Taylor J, Mobbs CV. Resistance to diet-induced obesity is associated with increased proopiomelanocortin mRNA and decreased neuropeptide Y mRNA in the hypothalamus. Brain Res 1999;851:198–203.
[5] Brady LS, Smith MA, Gold PW, Herkenham M. Altered expression of hypothalamic neuropeptide mRNAs in food-restricted and food-deprived rats. Neuroendocrinology 1990;52:441–7.
[6] Broberger C. Hypothalamic cocaine- and amphetamine-regulated transcript (CART) neurons: histochemical relationship to thyrotropin-releasing hormone, melanin-concentrating hormone, orexin/hypocretin and neuropeptide Y. Brain Res 1999;848:101–13.
[7] del Giudice EM, Santoro N, Cirillo G, D′Urso L, Di Toro R, Perrone L. Mutational screening of the CART gene in obese children: identifying a mutation (Leu34Phe) associated with reduced resting energy expenditure and cosegregating with obesity phenotype in a large family. Diabetes 2001;50:2157–60.
[8] Douglass J, Daoud S. Characterization of the human cDNA and genomic DNA encoding CART: a cocaine- and amphetamine-regulated transcript. Gene 1996;169:241–5.
[9] Druce M, Bloom SR. Central regulators of food intake. Curr Opin Clin Nutr Metab Care 2003;6:361–7.
[10] Eisenstein J, Greenberg A. Ghrelin: update. Nutr Rev 2003;61:101–4.
[11] Farley C, Cook JA, Spar BD, Austin TM, Kowalski TJ. Meal pattern analysis of diet-induced obesity in susceptible and resistant rats. Obes Res 2003;11:845–51.
[12] Hagan MM, Rushing PA, Schwartz MW, et al. Role of the CNS melanocortin system in the response to overfeeding. J Neurosci 1999;19:2362–7.
[13] Haynes WG, Morgan DA, Djalali A, Sivitz WI, Mark AL. Interactions between the melanocortin system and leptin in control of sympathetic nerve traffic. Hypertension 1999;33:542–7.
[14] Hewson AK, Tung LY, Connell DW, Tookman L, Dickson SL. The rat arcuate nucleus integrates peripheral signals provided by leptin, insulin, and a ghrelin mimetic. Diabetes 2002;51:3412–9.
[15] Huang XF, Lin S, Zhang R. Upregulation of leptin receptor mRNA expression in obese mouse brain. Neuroreport 1997;8:1035–8.
[16] Jequier E. Leptin signaling, adiposity, and energy balance. Ann N Y Acad Sci 2002;967:379–88.
[17] Kristensen P, Judge ME, Thim L, et al. Hypothalamic CART is a new anorectic peptide regulated by leptin. Nature 1998;393:72–6.
[18] Levin BE, Dunn-Meynell AA. Defense of body weight depends on dietary composition and palatability in rats with diet-induced obesity. Am J Physiol Regul Integr Comp Physiol 2002;282:R46–54.
[19] Levin BE, Dunn-Meynell AA. Dysregulation of arcuate nucleus preproneuropeptide Y mRNA in diet-induced obese rats. Am J Physiol 1997;272:R1365–70.
[20] Levin BE, Dunn-Meynell AA. Reduced central leptin sensitivity in rats with diet-induced obesity. Am J Physiol Regul Integr Comp Physiol 2002;283:R941–8.
[21] Levin BE. Arcuate NPY neurons and energy homeostasis in diet-induced obese and resistant rats. Am J Physiol 1999;276:R382–7.
[22] Li HY, Hwang HW, Hu YH. Functional characterizations of cocaine- and amphetamine-regulated transcript mRNA expression in rat hypothalamus. Neurosci Lett 2002;323:203–6.
[23] Lin S, Storlien LH, Huang XF. Leptin receptor, NPY, POMC mRNA expression in the diet-induced obese mouse brain. Brain Res 2000;875:89–95.
[24] Mercer JG, Speakman JR. Hypothalamic neuropeptide mechanisms for regulating energy balance: from rodent models to human obesity. Neurosci Biobehav Rev 2001;25:101–16.
[25] Murphy B, Nunes CN, Ronan JJ, et al. Melanocortin mediated inhibition of feeding behavior in rats. Neuropeptides 1998;32:491–7.
[26] Murray CD, Kamm MA, Bloom SR, Emmanuel AV. Ghrelin for the gastroenterologist: history and potential. Gastroenterology 2003;125:1492–502.
[27] Robson AJ, Rousseau K, Loudon AS, Ebling FJ. Cocaine and amphetamine-regulated transcript mRNA regulation in the hypothalamus in lean and obese rodents. J Neuroendocrinol 2002;14:697–709.
[28] Rohner-Jeanrenaud F, Craft LS, Bridwell J, et al. Chronic central infusion of cocaine- and amphetamine-regulated transcript (CART 55-102): effects on body weight homeostasis in lean and high-fat-fed obese rats. Int J Obes Relat Metab Disord 2002;26:143–9.
[29] Sainsbury A, Cooney GJ, Herzog H. Hypothalamic regulation of energy homeostasis. Best Pract Res Clin Endocrinol Metab 2002;16:623–37.
[30] Schrauwen P, Westerterp KR. The role of high-fat diets and physical activity in the regulation of body weight. Br J Nutr 2000;84:417–27.
[31] Schwartz MW, Woods SC, Porte Jr D, Seeley RJ, Baskin DG. Central nervous system control of food intake. Nature 2000;404:661–71.
[32] Smith Y, Kieval J, Couceyro PR, Kuhar MJ. CART peptide-immunoreactive neurones in the nucleus accumbens in monkeys: ultrastructural analysis, colocalization studies, and synaptic interactions with dopaminergic afferents. J Comp Neurol 1999;407:491–511.
[33] Surwit RS, Wang S, Petro AE, et al. Diet-induced changes in uncoupling proteins in obesity-prone and obesity-resistant strains of mice. Proc Natl Acad Sci USA 1998;95(7):4061–5.
[34] Vrang N, Tang-Christensen M, Larsen PJ, Kristensen P. Recombinant CART peptide induces c-Fos expression in central areas involved in control of feeding behaviour. Brain Res 1999;818:499–509.
[35] Wang J, Akabayashi A, Dourmashkin J, et al. Neuropeptide Y in relation to carbohydrate intake, corticosterone and dietary obesity. Brain Res 1998;802:75–88.
[36] Wang Z, Zhou YT, Kakuma T, et al. Leptin resistance of adipocytes in obesity: role of suppressors of cytokine signaling. Biochem Biophys Res Commun 2000;277:20–6.

[37] West DB, Waguespack J, McCollister S. Dietary obesity in the mouse: interaction of strain with diet composition. Am J Physiol 1995;268(3 Pt 2):R658–65.
[38] Widdowson PS, Upton R, Buckingham R, Arch J, Williams G. Inhibition of food response to intracerebroventricular injection of leptin is attenuated in rats with diet-induced obesity. Diabetes 1997;46(11):1782–5.
[39] Ziotopoulou M, Mantzoros CS, Hileman SM, Flier JS. Differential expression of hypothalamic neuropeptides in the early phase of diet-induced obesity in mice. Am J Physiol Endocrinol Metab 2000;279:E838–45.