ORIGINAL

Corticotropin-releasing hormone (CRH) transgenic mice display hyperphagia with increased Agouti-related protein mRNA in the hypothalamic arcuate nucleus

Shuichi Nakayama¹⁾, Mitsuru Nishiyama¹⁾, Yasumasa Iwasaki¹⁾, Masayuki Shinahara¹⁾, Yasushi Okada²⁾, Masayuki Tsuda²⁾, Mizuho Okazaki¹⁾, Makoto Tsugita¹⁾, Takafumi Taguchi¹⁾, Shinya Makino¹⁾, Mary P Stenzel-Poore³⁾, Kozo Hashimoto¹⁾ and Yoshio Terada¹⁾

¹⁾ Department of Endocrinology, Metabolism and Nephrology, Kochi Medical School, Kochi University, Nankoku 783-8505, Japan

²⁾ Department of Laboratory Animal Science, Kochi Medical School, Kochi University, Nankoku 783-8505, Japan

³⁾ Department of Molecular Microbiology and Immunology, Oregon Health Science University, Portland, OR 97201, USA

Abstract. Although glucocorticoid-induced hyperphagia is observed in the patients with glucocorticoid treatment or Cushing's syndrome, its molecular mechanism is not clear. We thus explored the expression of neuropeptide mRNAs in the hypothalamus related to appetite regulation in CRH over-expressing transgenic mice (CRH-Tg), a model of Cushing's syndrome. We measured food intake, body weight (including body fat weight) and plasma corticosterone levels in CRH-Tg and their wild-type littermates (WT) at 6 and 14 weeks old. We also examined neuropeptide Y (NPY), proopiomelanocortin (POMC) and Agouti-related protein (AgRP) mRNAs in the arcuate nucleus (ARC) using *in situ* hybridization. Circulating corticosterone levels in CRH-Tg were markedly elevated at both 6 and 14 weeks old. Body fat weight in CRH-Tg was significantly increased at 14 weeks old, which is considered as an effect of chronic glucocorticoid excess. At both 6 and 14 weeks old, CRH-Tg mice showed significant hyperphagia compared with WT (14w old: WT 3.9±0.1, CRH-Tg 5.1±0.7 g/day, $p<0.05$). Unexpectedly, NPY mRNA levels in CRH-Tg were significantly decreased at 14 weeks old (WT: 1571.5±111.2, CRH-Tg: 949.1±139.3 dpm/mg, $p<0.05$), and there were no differences in POMC mRNA levels between CRH-Tg and WT. On the other hand, AgRP mRNA levels in CRH-Tg were significantly increased compared with WT at both ages (14w old: WT 365.6±88.6, CRH-Tg 660.1±87.2 dpm/mg, $p<0.05$). These results suggest that glucocorticoid-induced hyperphagia is associated with increased hypothalamic AgRP. Our results also indicate that hypothalamic NPY does not have an essential role in the increased food intake during glucocorticoid excess.

Key words: Cushing's syndrome, Appetite, Obesity, Glucocorticoid, Agouti-related protein

THE PREVALENCE of overweight and obesity is increasing at a progressive rate over these days, and it is estimated that about one fourth of the world's adult population has the metabolic syndrome [1-3]. Excess weight causes increased risks of hypertension, impaired glucose tolerance, dyslipidemia and coronary heart disease, all of which are also essential components of metabolic syndrome. These abnormalities in the metabolic syndrome are associated with endothelial dysfunction and atherosclerosis, and increase the risk for diabetes

mellitus as well as vascular morbidity and mortality [4, 5]. While lifestyle management programs are useful for weight loss, a safe and effective appetite-suppressive agent could provide additional benefit for weight maintenance [6].

Cushing's syndrome is caused by chronic exposure to excess glucocorticoid hormones, either produced by the adrenal cortex or administered exogenously [7]. The chronic increase in circulating glucocorticoid could induce central obesity, hypertension, diabetes mellitus, dyslipidemia, fatty liver, which are also observed in the metabolic syndrome. Local glucocorticoid excess could also occur in the abdominal adipose tissue, when glucocorticoid-activating enzyme (11 β -hydroxysteroid dehydrogenase type 1) is overexpressed [8, 9]. Because the diagnostic features of metabolic syndrome are very

Received Nov. 29, 2010; Accepted Feb. 4, 2011 as K10E-370

Released online in J-STAGE as advance publication Mar. 5, 2011

Correspondence to: Mitsuru Nishiyama, M.D., Ph.D., Department of Endocrinology, Metabolism and Nephrology, Kochi Medical School, Kochi University, Kohasu, Oko-cho, Nankoku city, Kochi 783-8505, Japan. E-mail: nisiyamm@kochi-u.ac.jp

similar to those of Cushing's syndrome, it is proposed that glucocorticoid excess contributes to the pathogenesis of both states [10-12]. Obesity in Cushing's syndrome is characterized by increased appetite [13] and body weight, especially in visceral fat-dominant obesity with reduced lean mass.

There are several reports on the role of glucocorticoid-induced hyperphagia; central administration of glucocorticoid increases both food intake and hypothalamic neuropeptide Y (NPY) mRNA levels [14]. Furthermore, adrenalectomy reverses obese phenotype in ob/ob mice with a decrease in Agouti-related protein (AgRP) and an increase in proopiomelanocortin (POMC) mRNA levels in the hypothalamus [15]. These findings suggest that the central neuropeptides could be involved in the glucocorticoid-induced hyperphagia. Recently, Christ-Crain *et al.* has reported that systemic administration of glucocorticoid induced visceral obesity with increased hypothalamic adenosine monophosphate-dependent kinase (AMPK) activity [16]. However, the molecular mechanism of glucocorticoid-induced hyperphagia is not completely understood.

In this study, we tried to address this issue using the corticotropin-releasing hormone (CRH) transgenic overexpression mice (CRH-Tg), an animal model of Cushing's syndrome with glucocorticoid excess [17]. We examined the mRNA expression levels of the appetite-related neuropeptides such as NPY, POMC and AgRP in the hypothalamic arcuate nucleus (ARC) both in CRH-Tg and wild-type littermates. We found that CRH-Tg showed hyperphagia with an increase in hypothalamic AgRP mRNA, but unexpectedly, a decrease in NPY mRNA levels. The present results suggest that AgRP, but not NPY, could be responsible for the glucocorticoid-induced hyperphagia.

Materials and Methods

Animals

We used CRH-Tg, an animal model of Cushing's syndrome, which was characterized previously [17], and also their wild-type littermates (WT). All the experiments were carried out using male mice. All mice were

maintained under controlled conditions of light (lights on, 0600-1800 h) at 24°C in a humidity-controlled room, and are allowed free access to standard chow and drinking water. All procedures were approved by the animal committee of Kochi Medical School.

Evaluation of the metabolic parameters in CRH-Tg mice

Body weights of CRH-Tg and WT littermates were measured once a week, until the end of the experiment. Their food weights were also measured, and mean daily food intake corrected by their body weight during the final week before decapitation were determined as an index of food consumption. For the determination of blood hormone levels, CRH-Tg mice and their WT littermates (6 or 14 weeks old, n=6-8 in each group) were decapitated between 0900-1200 h. Trunk blood was collected for measuring plasma corticosterone and insulin levels using commercially available RIA/ELISA kits (corticosterone RIA kit, MP Biomedicals, OH, USA, and insulin ELISA kit, Morinaga Inc., Japan). Blood glucose levels were also determined by the enzymic method (FreeStyle: Nipro Inc., Japan). In both groups of mice, the thymus, adrenal glands, and epididymal fat were obtained for weighing.

In situ hybridization

After decapitation, the brain of each mouse was quickly removed and frozen by immersion in 2 methyl butane at -30 °C, and then stored at -80 °C until the tissue was sectioned on a cryostat. Frozen brain tissue was cut coronally in 20-μm thick sections for the *in situ* hybridization of the arcuate nucleus (ARC). The sections were taken from the following sites: ARC 1.40-1.90 mm posterior from the bregma. The sections were then thaw-mounted and air-dried on gelatin-coated slides, and were stored at -80 °C prior to the *in situ* hybridization.

For NPY, POMC and AgRP, a synthetic 48-base or 45-base oligodeoxynucleotide probe for each peptide mRNA was used (Table 1). The probes were labeled with [α -³⁵S] dATP (>1000 Ci/mmol, Perkin Elmer Inc., Waltham, MA, USA) using terminal deoxynucleotidyl transferase (25 units/mL, Boehringer-Mannheim Biochemicals, Indianapolis, IN, USA) and tailing buf-

Table 1 Antisense oligonucleotides used for *in situ* hybridization

mRNA	Oligonucleotide sequence
NPY	5'- GTC CTC TGC TGG CGC GTC CTC GCC CGG ATT GTC CGG CTT GGA GGG GTA - 3'
POMC	5'- GCC CAC CGG CTT GCC CCA GCG GAA GTG CTC CAT GGA GTA GGA GCG CTT - 3'
AgRP	5'- TGC AGC AGA ACT TCT TCT GCT CGG TCT GCA GTT GTC TTC TTG AGG - 3'

fer (Bethesda Research Laboratory, Bethesda, MD, USA). Then the probes were purified by phenol-chloroform extraction.

Hybridization procedures were described previously [18]. In brief, sections were fixed in 4% formaldehyde, and then treated with 0.25% acetic anhydride in 0.1 M triethanolamine/ 0.9% saline (pH 8.0) for 10 min to reduce nonspecific hybridization of the probe. The sections were then dehydrated in increasing concentrations of ethanol, and delipidated with chloroform for 5 min, rinsed in ethanol, and air-dried. Sections were hybridized overnight at 37 °C with 5×10^5 cpm of labeled NPY, POMC or AgRP probe per section, and then washed four times (15 min each) with 2×SCC ($1 \times = 0.15$ M NaCl/ 0.015 M sodium citrate, pH 7.2) containing 50% formamide at 40 °C, followed by two 30-min rinses with 1×SCC at 25 °C. Finally, the slides were dehydrated for 1 min in ascending alcohols (50, 70, 90 and 95 % ethanol containing 300 mM ammonium acetate, and 100 % ethanol). The procedures for imaging and quantitative analyses were described previously [18].

Statistics

All data are expressed as the mean $\pm$ SEM. Analyses were performed using ANOVA followed by Fisher's PLSD test, and $p < 0.05$ was determined as statistical significance.

Results

CRH-Tg shows phenotype of glucocorticoid excess

In CRH-Tg, plasma corticosterone levels were markedly elevated compared with that in WT littermates at both 6 and 14 weeks old (Fig. 1). Hyperinsulinemia was also observed in CRH-Tg, which could be secondarily induced by glucocorticoid excess [19]. Additionally, blood glucose levels in CRH-Tg were lower at 6 weeks old but higher at 14 weeks old compared with those in WT littermates (6w old: WT 117 ± 5.5 , CRH-Tg 85.7 ± 4.6 mg/dL, $p < 0.05$; 14w old: WT 78.8 ± 5.0 , CRH-Tg 125.6 ± 21.1 mg/dL, $p < 0.05$), although persistent glucocorticoid excess was observed. CRH-Tg also showed atrophic thymus and hypertrophic adrenal glands compared with WT littermates, reflecting the actual existence of ACTH-induced hyperadrenocorticism [19].

Body weight, but not epididymal fat weight, in CRH-Tg was significantly lower at 6 weeks old, reflecting glucocorticoid-induced growth retardation [7]. By aging, epididymal fat weight in CRH-Tg was

significantly higher at 14 weeks old, probably due to an effect of chronic glucocorticoid excess (Fig. 2).

CRH-Tg shows hyperphagia with glucocorticoid excess

CRH-Tg showed marked hyperphagia compared with WT littermates at both 6 and 14 weeks old after correction by their body weight (Fig. 3). Absolute food consumption in CRH-Tg was significantly higher at 14 weeks old.

Hypothalamic mRNA levels of NPY, POMC and AgRP in CRH-Tg

NPY mRNA levels in the ARC were similar between CRH-Tg and WT at 6 weeks old, but, unexpectedly, those in CRH-Tg were significantly lower compared with those in WT littermates at 14 weeks old (Fig. 4). There were no differences in POMC mRNA levels in the ARC between WT and CRH-Tg at both ages (Fig. 5). In contrast, AgRP mRNA levels in the ARC in CRH-Tg were significantly higher compared with those in WT littermates at both 6 and 14 weeks (Fig. 6). NPY and AgRP mRNAs at 14 weeks old were tendency to decrease compared with those at 6 weeks old, which correspond to aging-related changes in hypothalamic neuropeptides [20, 21].

Discussion

In this study, we aimed to identify the hypothalamic neuropeptide(s), which is involved in the development of glucocorticoid-induced hyperphagia. The obtained data showed that CRH-Tg displayed hyperphagia with increased AgRP mRNA levels in the hypothalamic ARC. Obesity in the patients with glucocorticoid treatment or Cushing's syndrome is characterized by increased energy intake [13]. Recent neuroendocrinological studies have clarified the involvement of a number of hypothalamic neuropeptides in appetite control, among which NPY, POMC-related peptide(s) and AgRP are assumed to play important roles [22, 23]. Indeed, in an animal model of Cushing's syndrome, we observed significant increase in the expression of hypothalamic AgRP. These results support the idea that AgRP, an orexigenic neuropeptide, could be responsible for the increased appetite during glucocorticoid excess. Although CRH is known to be an anorexigenic neuropeptide [24, 25], CRH-Tg displayed hyperphagia and obesity, suggesting that glucocorticoid excess overcomes the effect of CRH in the

Fig. 1 Plasma concentrations of corticosterone (A) and insulin (B) in WT littermates (control; white bars) and CRH-Tg (black bars) at 6 and 14 weeks old ($n=6-8$, in each group). Values are means $\pm$ SEM. * $p < 0.05$ vs. WT littermates.

Fig. 2 Body weight (A) and epididymal fat/body weight ratio (B) in WT littermates (control; white bars) and CRH-Tg (black bars) at 6 and 14 weeks old ($n=6-8$, in each group). Values are means $\pm$ SEM. * $p < 0.05$ vs. WT littermates.

Fig. 3 Mean daily food intake (A) and mean daily food intake corrected by body weight (B) in WT littermates (control; white bars) and CRH-Tg (black bars) at 6 and 14 weeks old ($n=6-8$, in each group). Values are means $\pm$ SEM. * $p < 0.05$ vs. WT littermates.

Fig. 4 NPY mRNA levels in the ARC (A) and film autoradiography of *in situ* hybridization for NPY mRNA in the ARC (B) in WT littermates (control; white bars) and CRH-Tg (black bars) at 6 and 14 weeks old (n=6-8, in each group). Values are means $\pm$ SEM. * p <0.05 vs. WT littermates.

Fig. 5 POMC mRNA levels in the ARC (A) and film autoradiography of *in situ* hybridization for POMC mRNA in the ARC (B) in WT littermates (control; white bars) and CRH-Tg (black bars) at 6 and 14 weeks old (n=6-8, in each group). Values are means $\pm$ SEM.

Fig. 6 AgRP mRNA levels in the ARC (A) and film autoradiography of *in situ* hybridization for AgRP mRNA in the ARC (B) in WT littermates (control; white bars) and CRH-Tg (black bars) at 6 and 14 weeks old (n=6-8, in each group). Values are means $\pm$ SEM. * p <0.05 vs. WT littermates

animal model of Cushing's syndrome. Further studies (*e. g.* effect of adrenalectomy) are necessary to distinguish glucocorticoid effect from central CRH over-expression in CRH-Tg.

Melanocortin 4 receptor (MC4R) plays a crucial role in the regulation of feeding behavior, as observed with both genetic and pharmacological technologies [26, 27]. MC4Rs on the hypothalamic neurons receive both agonistic and antagonistic signals. α -melanocyte-stimulating hormone (α -MSH) derived from the POMC gene stimulates the receptors and suppresses food intake with subsequent weight loss [28]. In contrast, AgRP, coexpressed in NPY neurons, is an endogenous antagonist of α -MSH and it promotes food intake and positive energy balance [29]. In the previous studies, adrenocortical insufficiency induced by either adrenalectomy or POMC-deficiency reduced the expression of hypothalamic AgRP mRNA, which were reversed by glucocorticoid replacement [15, 30, 31]. Our obtained data are in accordance with the reports, showing that the expression of hypothalamic AgRP mRNA was significantly higher in CRH-Tg with increased food intake. Since the expression levels of hypothalamic POMC were not changed, and those of hypothalamic NPY were rather decreased, we assumed that AgRP could be a key molecule in the glucocorticoid-induced hyperphagia and central obesity. Shimizu *et al.* have reported that glucocorticoid increased both AgRP gene expression and AMPK phosphorylation in hypothalamic culture, suggesting that glucocorticoid increased AgRP mRNA involving AMPK [32]. However, Claret *et al.* showed that AgRP mRNA levels in the ARC did not differ between mice lacking AMPK α 2 in the AgRP neurons and their WT littermates [33]. Altogether, it is absolutely necessary to clarify the molecular mechanism(s) how glucocorticoid regulates AgRP gene expression *in vivo*.

It has been generally believed that NPY is one of the key molecule in glucocorticoid-induced hyperphagia [14, 32, 34], unexpectedly, however, we observed hypothalamic NPY mRNA expressions in CRH-Tg were rather decreased. This is of interest because NPY

and AgRP are known to be co-expressed within the same neuronal cells of the ARC [35]. It is also recognized that AgRP is regulated more dynamically compared with NPY [15, 31], supporting the different regulation of NPY and AgRP gene within the same cell. Furthermore, it has been reported the possibility that CRH negatively regulates hypothalamic NPY expressions [36], which could explain the decrease of NPY mRNA in CRH-Tg. Intracerebroventricular injection of CRH will clarify direct effect of CRH on these hypothalamic neuropeptides. Our present results also indicate that NPY is not essential in the regulation of glucocorticoid-induced hyperphagia.

It is well known that glucocorticoid exerts negative effect on POMC mRNA in the anterior pituitary gland, but its effect on hypothalamic POMC expression is not completely understood. Indeed, the effects of adrenalectomy and/or glucocorticoid replacement on the expression of POMC mRNA in the ARC are contradictory [15, 30, 31]. In our current study, we could not find differences in hypothalamic POMC mRNA levels between CRH-Tg and WT littermates.

Considering the fact that glucocorticoid excess significantly contributes to the pathogenesis not only in Cushing's syndrome but also in the metabolic syndrome [10-12], it is possible that hypothalamic neuropeptide(s) associated with glucocorticoid-induced hyperphagia play a role in the development of simple obesity. Accumulating evidences and our present study suggest that AgRP could be a key molecule in the glucocorticoid-induced hyperphagia and obesity. In addition, further studies will clarify the roles of other neuropeptides such as ghrelin, melanin-concentrating hormone, orexin, and other factor(s) in glucocorticoid-mediated appetite regulation.

Acknowledgement

This work was supported in part by Health and Labour Science Research Grants (Research on Measures for Intractable Diseases) from the Ministry of Health, Labour and Welfare of Japan.

References

1. Athyros VG, Ganotakis ES, Ekisaf M, Mikhailidis DP (2005) The prevalence of the metabolic syndrome using the National Cholesterol Education Program and International Diabetes Federation definitions. *Curr Med Res Opin* 21: 1157-1159.
2. Matsushita Y, Takahashi Y, Mizoue T, Inoue M, Noda M,

- Tsugane S (2008) Overweight and obesity trends among Japanese adults: a 10-year follow-up of the JPHC Study. *Int J Obes (Lond)* 32: 1861-1867.
3. Kolovou GD, Anagnostopoulou KK, Salpea KD, Mikhailidis DP (2007) The prevalence of metabolic syndrome in various populations. *Am J Med Sci* 333: 362-371.
 4. Ford ES (2005) Risks for all-cause mortality, cardiovascular disease, and diabetes associated with the metabolic syndrome: a summary of the evidence. *Diabetes Care* 28: 1769-1778.
 5. Smith SCJ (2007) Multiple risk factors for cardiovascular disease and diabetes mellitus. *Am J Med* 120: S3-S11.
 6. Halford JCG, Boyland EJ, Blundell JE, Kirkham TC, Harrold JA (2010) Pharmacological management of appetite expression in obesity. *Nat Rev Endocrinol* 6: 255-269.
 7. Arnaldi G, Angeli A, Atkinson AB, Bertagna X, Cavagnini F, Chrousos GP, Fava GA, Findling JW, Gaillard RC, Grossman AB, Kola B, Lacroix A, Tancini T, Mantero F, Newell-Price F, Nieman LK, Sonino N, Vance ML, Giustina A, Boscaro M (2003) Diagnosis and complications of Cushing's syndrome: a consensus statement. *J Clin Endocrinol Metab* 88: 5593-5602.
 8. Masuzaki H, Paterson J, Shinyama H, Morton NM, Seckl JR, Flier JS (2001) A transgenic model of visceral obesity and the metabolic syndrome. *Science* 294: 2166-2170.
 9. Tomlinson JW, Walker EA, Bujalska IJ, Draper N, Lavery GG, Cooper MS, Hewison M, Stewart PM (2004) 11 β -hydroxysteroid dehydrogenase type 1: a tissue-specific regulator of glucocorticoid response. *Endocrine Reviews* 25: 831-866.
 10. Bujalska IJ, Kumar S, Stewart PM (1997) Does central obesity reflect "Cushing's disease for the omentum"? *Lancet* 349: 1210-1213.
 11. Pasquali R, Viccattini V, Cacciari M, Pagotto U (2006) The hypothalamic-pituitary-adrenal axis activity in obesity and the metabolic syndrome. *Ann NY Acad Sci* 1083: 111-128.
 12. Anagnostis P, Athyros VG, Tziomalos K, Karagiannis A, Mikhailidis DP (2009) The pathogenetic role of cortisol in the metabolic syndrome: a hypothesis. *J Clin Endocrinol Metab* 94: 2692-2701.
 13. Tataranni PA, Larson DE, Snitker S, Young JB, Flatt JP, Ravussin E (1996) Effects of glucocorticoids on energy metabolism and food intake in humans. *Am J Physiol* 271: E317-E325.
 14. Zakrzewska KE, Cusin I, Stricker-Krongrad A, Boss O, Ricquier D, Jeanrenaud B, Rohner-Jeanrenaud F (1999) Induction of obesity and hyperleptinemia by central glucocorticoid infusion in the rat. *Diabetes* 48: 365-370.
 15. Makimura H, Mizuno TM, Roberts J, Silverstein J, Beasley J, Mobbs CV (2000) Adrenalectomy reverses obese phenotype and restores hypothalamic melanocortin tone in leptin-deficient ob/ob mice. *Diabetes* 49: 1917-1923.
 16. Christ-Crain M, Kola B, Lolli F, Fekete C, Seboek D, Wittmann G, Feltrin D, Igreja SC, Ajodha S, Harvey-White J, Kunos G, Muller B, Pralong F, Aubert G, Arnaldi G, Giacchetti G, Boscaro M, Grossman AB, Korbonits M (2008) AMP-activated protein kinase mediates glucocorticoid-induced metabolic changes: a novel mechanism in Cushing's syndrome. *FASEB J* 22: 1672-1683.
 17. Stenzel-Poore MP, Cameron VA, Vaughan J, Sawchenko PE, Vale WW (1992) Development of Cushing's syndrome in corticotropin-releasing factor transgenic mice. *Endocrinology* 130: 3378-3386.
 18. Nishiyama M, Makino S, Asaba K, Hashimoto K (1999) Leptin effects on the expression of type-2 CRH receptor mRNA in the ventromedial hypothalamus in the rat. *J Neuroendocrinol* 11: 307-314.
 19. Shinahara M, Nishiyama M, Iwasaki Y, Nakayama S, Noguchi T, Kambayashi M, Okada Y, Tsuda M, Stenzel-Poore MP, Hashimoto K, Terada Y (2009) Plasma adiponectin levels are increased despite insulin resistance in Corticotropin-releasing hormone transgenic mice, an animal model of Cushing syndrome. *Endocr J* 56: 879-886.
 20. Kaneda T, Makino S, Nishiyama M, Asaba K, Hashimoto K (2001) Differential neuropeptide response to starvation with aging. *J Neuroendocrinol* 13: 1066-1075.
 21. Wolden-Hanson T, Marck BT, Matsumoto AM (2004) Blunted hypothalamic neuropeptide gene expression in response to fasting, but preservation of feeding response to AgRP in aging male Brown Norway rats. *Am J Physiol* 287: R138-R146.
 22. Flier JS (2004) Obesity wars: Molecular progress confronts an expanding epidemic. *Cell* 116: 337-350.
 23. Morton GJ, Cummings DE, Baskin DG, Barsh GS, Schwartz MW (2006) Central nervous system control of food intake and body weight. *Nature* 443: 289-295.
 24. Arase K, York DA, Shimizu H, Shargil NS, Bray GA (1988) Effect of corticotropin-releasing factor on food intake and brown adipose tissue thermogenesis in rats. *Am J Physiol* 255: E255-E259.
 25. Glowa J, Gold PW (1991) Corticotropin-releasing hormone produces profound anorexigenic effects in the rhesus monkey. *Neuropeptides* 18: 55-61.
 26. Huszar D, Lynch CA, Fairchild-Huntress V, Dunmore JH, Fang Q, Berkemeier LG, Gu W, Kesterson RA, Boston BA, Cone RD (1997) Targeted disruption of the melanocortin-4 receptor results in obesity in mice. *Cell* 88: 131-141.
 27. Cone RD (1999) The central melanocortin system and energy homeostasis. *Trends Endocrinol Metab* 10: 211-216.
 28. Fan W, Boston BA, Kesterson RA, Hruby VJ, Cone RD

- (1997) Role of melanocortinergic neurons in feeding and the agouti obesity syndrome. *Nature* 385: 165-168.
29. Ollmann MM, Wilson BD, Yang YK, Kerns JA, Chen Y, Gantz I, Barsh GS (1997) Antagonism of central melanocortin receptors in vitro and in vivo by agouti-related protein. *Science* 278: 135-138.
 30. Savontaus E, Conwell IM, Wardlaw SL (2002) Effects of adrenalectomy on AGRP, POMC, NPY and CART gene expression in the basal hypothalamus of fed and fasted rats. *Brain Res* 958: 130-138.
 31. Coll AP, Challis BG, Lopez M, Piper S, Yeo GSH, O'Rahilly S (2005) Proopiomelanocortin-deficient mice are hypersensitive to the adverse metabolic effects of glucocorticoids. *Diabetes* 54: 2269-2276.
 32. Shimizu H, Arima H, Watanabe M, Goto M, Banno R, Sato I, Ozaki N, Nagasaki H, Oiso Y (2008) Glucocorticoids increase neuropeptide Y and Agouti-related peptide gene expression via adenosine monophosphate-activated protein kinase signaling in the arcuate nucleus of rats. *Endocrinology* 149: 4544-4553.
 33. Claret M, Smith MA, Batterham RI, Selman C, Choudhury AI, Fryer LG, Clements M, Al-Qassab H, Heffron H, Xu AW, Speakman JR, Barsh GS, Violette B, Vaulont S, Ashford MI, Carling D, Withers DJ (2007) AMKP is essential for energy homeostasis regulation and glucose sensing by POMC and AgRP neurons. *J Clin Invest* 117: 2325-2336.
 34. Watanabe Y, Akabayashi A, McEwen BS (1995) Adrenal steroid regulation of neuropeptide Y (NPY) mRNA: differences between dentate hilus and locus coeruleus and arcuate nucleus. *Mol Brain Res* 28: 135-140.
 35. Hahn TM, Breininger JF, Baskin DG, Schwartz MW (1998) Coexpression of Agrp and NPY in fasting-activated hypothalamic neurons. *Nat Neurosci* 1: 271-272.
 36. Bchini-Hooft van Huijsduijnen OB, Rohner-Jeanrenaud F, Jeanrenaud B (1993) Hypothalamic neuropeptide Y messenger ribonucleic acid levels in pre-obese and genetically obese (fa/fa) rats: potential regulation thereof by corticotropin-releasing factor. *J Neuroendocrinol* 5: 381-386.