Gene Expression and the Control of Food Intake by Hypothalamic POMC/CART Neurons

Jennifer W. Hill*

Center for Diabetes and Endocrine Research, University of Toledo, College of Medicine, USA

Abstract: Neurons that express pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) in the arcuate nucleus of the hypothalamus suppress feeding and increase energy expenditure in response to circulating adiposity signals such as leptin. Alterations in gene expression may lead to long term modification of this circuit and alterations in body weight. Therefore, understanding how gene expression in these neurons is controlled is crucial to forming a complete picture of the central management of energy balance. This review outlines the heterogeneity of arcuate POMC/CART neurons, describes our current understanding of CART and POMC gene transcription in these neurons, and suggests future directions for extending the field.

Keywords: POMC, CART, leptin, transcription, gene expression, obesity, energy balance.

INTRODUCTION

The arcuate nucleus (ARC) of the hypothalamus plays a key role in the control of food intake, containing opposing orexigenic and anorexigenic neuronal circuits. The latter are composed of neurons that express pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART). When activated, POMC/CART neurons signal to downstream neuronal pathways that suppress feeding and increase energy expenditure. Circulating adiposity signals such as leptin modulate POMC/CART neuronal activity and alter gene transcription in these neurons to coordinate energy homeostasis.

Leptin, a product of the OB (or LEP) gene, produced primarily in adipose tissue, plays an important role in food intake and body weight regulation. Defective leptin signaling due to either leptin deficiency, as in *ob/ob* mice, or mutation in the leptin receptor, as in *db/db* mice, leads to development of obesity [1-5]. Binding of leptin to its receptor induces activation of several signaling pathways, including the Janus kinase / Signal transducer and activator of transcription (JAK/STAT), Mitogen activated kinase-like protein (MAPK), Insulin responsive substrate 1 (IRS1), and Suppressor of cytokine signaling 3 (SOCS3) pathways, which mediate its effects. The JAK/STAT pathway serves as the primary leptin signal transduction pathway in the hypothalamus. In this signaling cascade, Jak2 activation leads to phosphorylation of the STAT3 transcription factor, which dimerizes and translocates to the nucleus where it regulates gene transcription [6, 7]. Alternatively, leptin signaling can alter neuronal activity without altering gene transcription though alternative pathways such as IRS- phosphoinositide 3-kinase (PI3K) signaling [8-10].

*Address correspondence to this author at the University of Toledo College of Medicine; Health Science Campus, 3000 Arlington Ave, Block Health Science Bldg., CeDER, Toledo, OH 43614-2598, USA;
Tel: 419-383-4183; Fax: 419-383-2871;
E-mail: Jennifer.Hill4@utoledo.edu

We have recently demonstrated that transient changes in the activity of POMC/CART neurons do not necessarily lead to long term alterations in body weight [9]. Nevertheless, permanent alteration of gene expression induced by adiposity signals may lead to long-term modification of the function of this circuit. Therefore, understanding how gene expression in these neurons is controlled is crucial to forming a complete picture of the central management of energy balance. This review will describe the current understanding of transcriptional control in these neurons and suggest future directions for extending the field.

COMPLEXITY IN THE NEURONAL POPULATION

POMC/CART neurons are found in the retrochiasmatic area (RCh) and throughout the rostrocaudal span of the ARC continuing caudally into the posterior periventricular nucleus (PVN) [11-18]. While these POMC/CART neurons are often referred to as being part of a single circuit, it is becoming clear that the population contains a significant amount of heterogeneity. To begin with, these neurons do not all project to the same downstream regions [19-22], suggesting that they serve different functions. In rats, both the retina and the suprachiasmatic nucleus project to the RCh [23], which in turn projects to the intergeniculate leaflet of the thalamus, suggesting involvement in the circadian system [24]. Additionally, neurons of the lateral RCh that express POMC/CART primarily project caudally to autonomic areas, including the dorsal vagal complex and the intermediolateral cell column (IML) [19, 22, 25]. On the other hand, the ARC projects extensively to the ventral part of the lateral septum, the bed nuclei of the stria terminalis (all subregions), the medial and periventricular parts of the preoptic area, the parvicellular parts of the PVN, the dorsomedial nucleus (DMN), the zona incerta and the lateral hypothalamic area (LHA) [26, 27]. Specifically, the more caudal POMC/CART cells project largely to hypothalamic centers like the PVN and to the external zone of the median eminence and the LHA [20, 21]. It is important to note that most of this anatomical data were gathered from the examination of rat

1876-5289/10

brains, and may or may not be directly applicable to mouse models, which are the subject of more recent genetic studies. As one example, POMC neurons are located both medially and ventrally in mouse ARC, in contrast to a predominantly lateral position in rat ARC. [8]

In addition to their projecting to different areas, subtypes can be identified within the POMC/CART population based on neurotransmitter or receptor expression. For instance, subsets of POMC neurons have been found to contain glutamate or gamma-aminobutyric acid (GABA) [28, 29]. In addition, besides the co-localization between CART and POMC, small fractions of CART neurons in the Arc have also been demonstrated to express dynorphin, neurotensin, or thyrotropin-releasing hormone mRNA [30]. Functional leptin receptors are found on approximately 35% of all POMC/CART neurons from the Rch and ARC of the mediobasal hypothalamus [31]. While leptin-induced excitation is observed throughout the rostrocaudal levels of the RCA and ARC, Williams and colleagues have recently shown that a higher percentage of leptin-excited POMC cells exist in the lateral division of the RCh and medial group of POMC cells in the Arc, such that 40-70% of POMC cells are excited by leptin in those regions [31]. This distribution correlates well with the involvement of LHA and PVN melanocortin-4 receptors in the acute effects of leptin on energy balance (see below). On the other hand, insulin-inhibited POMC cells are largely localized to the medial divisions of the RCh and rostromedial areas of the ARC, in agreement with the observed distribution of the insulin receptor. This pattern of insulin-inhibited POMC cells mirrors the location of "autonomic" POMC cells projecting to the dorsal vagal complex and IML. These findings suggest a segregation of insulin and leptin responses in arcuate POMC cells and a spatial separation of their downstream effects on intracellular signaling [31]. Thus, the receptor types expressed in POMC/CART neurons determine both the active signaling cascades and the genes transcribed in those neurons.

CART

The human CART prepropeptide gene encompasses approximately 1.9 kb and is composed of three exons and two introns [32]. Unlike humans, rodents have alternative splicing within exon 2 resulting in the production of two precursor proteins, one long (129 amino acids) and one short (116 amino acids) [11]. In humans, however, only the 116 amino acid (aa) polypeptide is found (hsCART). Newly synthesized prepro-CART molecules have a 27aa N-terminal hydrophobic signal sequence, which is deleted upon entry into the secretory pathway [11, 32]. These proteins are then processed by prohormone convertase (PC) while transiting through the Golgi complex to their final state within mature secretory granules [33].

Studies of human populations have implicated CART in the regulation of food intake. Obese members of a family in Italy had a missense mutation (Leu34Phe) in CART resulting in CART peptide deficiency due to mis-sorting, poor processing and secretion [34, 35]. Additional studies have linked polymorphisms in the 5' region of the CART gene with obesity [36, 37]. Complimentary findings have been produced through rodent studies [36]. In rats, CART central administration dose-dependently reduces food intake [38], and antibodies directed against CART peptide administered icv increase feeding [14]. Furthermore, CART mRNA in the ARC is decreased in food-deprived animals [14], and CART mRNA centrally delivered through a viral vector suppressed weight gain in rats on a high fat diet [39]. Finally, CART null mice develop increased food intake and obesity while on a high fat diet [40].

CART expression is also responsive to leptin levels. Mice lacking endogenous leptin or leptin receptors show reduced CART expression, while CART mRNA levels in the rat ARC are increased by administration of leptin [41]. Indeed, leptin receptors are found on CART-containing neurons in the ARC and other regions of the hypothalamus [18]. Interestingly, glucocorticoids may modulate the interaction between CART and leptin since CART expression is not changed by fasting or refeeding after adrenalectomy [42].

CART GENE TRANSCRIPTION

The CART promoter region contains several predicted binding sites for transcription factors such as CRE-binding protein (CREB), cJun, SP1, AP2 and STAT protein that are conserved across rats, mice and humans with the potential to regulate basal and stimulus-induced CART mRNA expression [43-47]. However, the number of studies investigating the action of these transcription factors in the control of hypothalamic CART gene expression in relation to energy homeostasis is limited. Investigation has shown that CREB protein affects CART gene transcription regulation [44-46, 48] by complexing with c-Jun, CREM, ATF-1, NFkB and CBP [49-55], and it has been found to mediate a forskolin-induced increase in CART mRNA levels via the protein kinase A (PKA) pathway in the rat nucleus accumbens [56]. It remains to be determined whether this pathway plays a major role in the response of POMC/CART neurons to altered energy availability.

The effect of lipopolysaccharide (LPS) on CART transcription has been investigated. LPS can induce anorexia by activating inflammatory cytokines [57] like interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor α (TNF-α). These cytokines may activate the AP1 family of transcription factors, thus altering CART mRNA expression. Intracerebroventricular or intraperitoneal administration of LPS causes a significant increase in arcuate CART mRNA levels, possibly due to an accompanying increase in corticosterone levels [58, 59]. Indeed, acute administration of corticosterone results in a more than 30% increase in the expression of CART in the nucleus accumbens [60]. Furthermore, adrenalectomized animals show a reduction in CART mRNA in the ARC that is reversed by hormone replacement [61, 62]. Thus, alterations in body weight as part of adaptation to stressors may be mediated by changes in CART gene transcription in the ARC.

The role of additional transcription factors in the regulation of CART gene expression in POMC/CART neurons would bear investigation. In particular, the existence of a STAT-binding motif in the CART promoter presents the very interesting possibility that the CART gene could be regulated directly by leptin's induction of the JAK/STAT pathway. The presence of an overlapping STAT/CRE/AP1 site in the CART promoter may indicate that STAT effects

on gene transcription can also be modified by other signaling pathways [44].

POMC

POMC is a polypeptide precursor that, once translated, is extensively modified to produce smaller, biologically-active fragments. The POMC gene consists of 3 exons covering 7.8kb in length. Although all 3 exons are transcribed, exon 1 contains only untranslated sequences, part of exon 2 codes for signaling peptide and the initial amino acids of the N-terminal peptide, and exon 3 codes for most of the translated RNA. Once translated, the peptides translocates through the membrane of the rough endoplasmic reticulum. It is then cleaved and trafficked as a secreted protein through the Golgi complex and eventually the secretory granules.

During trafficking, the POMC protein undergoes a series of posttranslational modifications through the actions of PC1/3 and PC2. POMC is partially cleaved to generate β-lipotrophin and pro-adrenocorticotropic hormone (ACTH). β-lipotrophin hormone (LPH) is then cleaved to form γ-LPH and β-endorphin and, in humans but not mice, γ-LPH is cleaved in turn to generate β-melanocyte stimulating hormone (MSH) [63, 64]. In the ARC [65-68], ProACTH is further cleaved by prohormone convertase 1/3 (PC1/3) to generate an N-terminal peptide and ACTH. In humans, three forms of γ-MSH are formed by additional cleavage of N-terminal POMC: γ_1-MSH, γ_2-MSH (not found in mice), and γ_3-MSH. ACTH is further cleaved to ultimately generate α-MSH and corticotrophin-like intermediate lobe peptide (CLIP). ACTH and the family of MSH peptides are known as melanocortins. The melanocortins mediate their effects in the CNS through two related G protein-coupled receptors, MC3R and MC4R.

Melancortins play an important role in the control of food intake and energy expenditure. Null POMC alleles result in obesity in both mice and humans [69]. In *Pomc* null mice, α-MSH was able to reduce food intake and body weight when centrally administered over a 3 day period, while ß-MSH, -LPH, and 3- and 2-MSH did not [70]. Indeed, α-MSH production is reduced during fasting [70]. In addition, deficiency in PC1/3, the enzyme required for α-MSH production, leads to increased body weight in humans [71, 72] and mice [73].

The interpretation of the mouse studies above in regards to human physiology is complicated by the fact that rodents lack the N-terminal cleavage site required to produce β-MSH, and it is therefore not an endogenous ligand in mice. Indeed, β-MSH appears to play an important role in body weight regulation in humans. Lee and colleagues [74] found that a missense mutation in the region encoding β -MSH co-segregated with obesity, and the mutation was shown to impair the ability of β-MSH to activate MC4R.

In contrast to the melanocortins, another product of the POMC gene, β-endorphin, a μ-opioid agonist, inhibits POMC cells [8, 75, 76] and increases food intake in rodents [77, 78]. Opioid antagonists increase activation of POMC neurons in the ARC, probably by removing tonic β-endorphin-mediated autoinhibition of POMC neurons [79].

POMC GENE TRANSCRIPTION

The relationship between POMC gene transcription and the control of energy balance has been extensively studied. In particular, the circulating adiposity factor leptin has been shown to modify POMC gene expression. For example, low leptin levels in fasted or *ob*/*ob* mice inhibit ARC POMC gene expression, [80] which can be reversed by leptin administration [81-83]. Evidence suggests that JAK/STAT signaling activated by leptin can directly modify POMC transcription through interaction with its promoter. The distal 5' sequence of the *POMC* gene, and in particular two regions, designated neuronal *POMC* enhancer 1 and 2 (nPE1 and nPE2), between −13 and −2 kb target gene expression to ARC neurons [84]. The former sequence contains a canonical STAT3-responsive element binding site. An additional, noncanonical STAT binding site has been found in the proximal enhancer region. STAT3 can increase *POMC* transcription by interacting with the site in this promoter region [84].

The Jak/STAT signaling pathway activated by leptin also co-ordinately regulates prohormone convertase 1/3 (PC1/3), which is crucial to POMC processing [85-87]. Food restriction suppresses PC1/3 levels and thus POMC-derived peptides such as α-MSH in the ARC, and administration of leptin reverses this response. [81, 88-90]. The human and mouse PC1/3 promoter share two putative STAT3 and E-box motifs [91, 92], although a third leptin-responsive STAT3 binding site is present in the human promoter [85]. These STAT sites have been implicated in leptin-mediated expression of PC1/3. Thus, leptin-initiated Jak/Stat signaling acts at multiple levels to reduce the production of POMC-derived peptides.

Downstream targets of another leptin-activated pathway also regulate POMC gene expression. The PI3K/Akt pathway has been implicated in the regulation of food intake and energy homeostasis by hypothalamic neurons [93-96]. Inhibition of PI3K attenuates the suppression of food intake by insulin as well as leptin [95, 96]. One downstream target of Akt is the forkhead transcriptional factor subfamily forkhead box O1 (FoxO1 or Fkhr) [97]. Activation of Akt phosphorylates FoxO1 and results in its exclusion from the nucleus and proteosomal degradation [97, 98], thereby inhibiting its action. Furthermore, expression of FOXO1 in the hypothalamus is decreased by insulin or leptin administration [97] in a PI3K dependent manner. FoxO1 has been reported to directly control POMC gene expression [83, 99], leading to a reduction in POMC mRNA. Interestingly, FoxO1 and STAT3 bind to adjacent sites in the promoter regions of *POMC* to regulate its expression [100], suggesting possible interaction between these two signaling pathways.

Another transcription factor that has been demonstrated to affect posttranslational processing of POMC products is nescient helix loop helix 2 (Nhlh2). Nhlh2 is a basic helix-loop-helix transcription factor that affects body weight through control of physical activity levels (3,7). Nhlh2 knockout (N2KO) mice display adult-onset obesity [101] and reduced production of POMC-derived peptides as a result of reduced POMC peptide processing of POMC. Indeed, a significant reduction in both PC1/3 and PC2 mRNA was found in the ARC of the N2KO mice [102]. Evidence suggests that Nhlh2 and leptin act coordinately to induce high levels of

PC1/3 gene transcription. STAT3 and Nhlh2 interact as a heterodimer on the PC1/3 promoter to mediate leptin-stimulated PC1/3 expression [103]. Thus, Nhlh2 acts cooperatively with STAT3 to induce PC1/3 expression following leptin stimulation.

Both androgens and estrogens have been found to affect POMC gene expression [104, 105]. For example, ovariectomy decreases POMC mRNA in the ARC [106], and this regulation is reversed by a short term replacement of estradiol [106]. Such nuclear steroid hormone receptors regulate the transcription of target genes by interacting with DNA response elements. Indeed, lower POMC levels are observed in mice lacking estrogen receptor α (ERα) [107, 108]. ERα mediates the classic transcriptional effects of estrogen, but can also be transcriptionally activated in a ligand-independent manner [109]. Leptin has been shown to activate ERα via the mitogen-activated protein kinase (MAPK) pathway *in vitro* in a ligand-independent manner [109]. These findings have implications for the widespread sexual dimorphism seen in the body weight phenotype of many transgenic studies targeting the POMC neuron [110-113].

Finally, POMC expression has been shown to be altered by 5-hydroxytryptamine (5-HT) signaling. POMC neurons in the ARC receive input from 5-HT-immunoreactive nerve terminals [114], and up to 80% of alpha-MSH expressing POMC neurons in the ARC express 5-HT2C receptors, with co-expression being greatest in the caudal ARC [115]. 5-HT2CR null mice develop hyperphagia, hyperactivity, and obesity and show attenuated responses to anorexigenic 5-HT drugs, which is normalized by re-expression of the receptor in POMC neurons alone [116]. Notably, infusion of a 5-HT2C receptor agonist significantly decreased POMC mRNA levels in both diet-induced obese and leptin deficient mice [117, 118]. The mechanism for this suppression remains to be characterized.

CLOSING REMARKS

Given that body weight control requires a coordinated modulation of food intake and energy expenditure over an extended time horizon, the gene expression of neurons regulating these functions must be carefully controlled. As this review has shown, our knowledge of the control of gene expression in POMC/CART neurons is incomplete and has tended to focus on well understood adiposity signals and transcription factors. No doubt far more complexity remains to be uncovered. In addition, however, studies of epigenetics as a method of long-term modulation of gene expression in these neurons are needed. In other tissues, the level of POMC expression is greatly influenced by the methylation pattern of the 5' promoter [119]. Should a similar process occur in POMC/CART neurons, the significance for the programming of body weight regulation in individuals and/or families could be profound. Therefore, the control of gene expression in POMC/CART neurons will continue be a critical area of investigation with important implications for the treatment of obesity in humans.

ACKNOWLEDGEMENTS

The author would like to thank Dr. Yong Xu for critical reading of this manuscript.

REFERENCES

[1] Zhang Y, Proenca R, Maffei M, *et al.* Positional cloning of the mouse obese gene and its human homologue. Nature 1994; 372: 425-32.

[2] Campfield LA, Smith FJ, Guisez Y, Devos R, Burn P. Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. Science 1995; 269: 546-9.

[3] Halaas JL, Gajiwala KS, Maffei M, *et al.* Weight-reducing effects of the plasma protein encoded by the obese gene. Science 1995; 269: 543-6.

[4] Pelleymounter MA, Cullen MJ, Baker MB, *et al.* Effects of the obese gene product on body weight regulation in ob/ob mice. Science 1995; 269: 540-3.

[5] Friedman Jm, Halaas JL. Leptin and the regulation of body weight in mammals. Nature 1998; 395: 763-70.

[6] Hegyi K, Fulop K, Kovacs K, Toth S, Falus A. Leptin-induced signal transduction pathways. Cell Biol Int 2004; 28: 159-69.

[7] Vaisse C, Halaas JL, Horvath CM, *et al.* Leptin activation of Stat3 in the hypothalamus of wild-type and ob/ob mice but not db/db mice. Nat Genet 1996; 14: 95-7.

[8] Cowley MA, Smart JL, Rubinstein M, *et al.* Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus. Nature 2001; 411: 480-4.

[9] Hill JW, Williams KW, Ye C, *et al.* Acute effects of leptin require PI3K signaling in hypothalamic proopiomelanocortin neurons in mice. J Clin Invest 2008; 118: 1796-805.

[10] Spanswick D, Smith MA, Groppi VE, Logan SD, Ashford ML. Leptin inhibits hypothalamic neurons by activation of ATP-sensitive potassium channels. Nature 1997; 390: 521-5.

[11] Douglass J, McKinzie AA, Couceyro P PCR differential display identifies a rat brain mRNA that is transcriptionally regulated by cocaine and amphetamine. J Neurosci 1995; 15: 2471-81.

[12] Koylu EO, Couceyro PR, Lambert PD, *et al.* Immunohistochemical localization of novel CART peptides in rat hypothalamus, pituitary and adrenal gland. J Neuroendocrinol 1997; 9: 823-33.

[13] Kristensen P, Judge ME, Thim L, *et al.* Hypothalamic CART is a new anorectic peptide regulated by leptin. Nature 1998; 393: 72-6.

[14] Lambert PD, Couceyro PR, McGirr KM, *et al.* CART peptides in the central control of feeding and interactions with neuropeptide Y. Synapse 1998; 29: 293-8.

[15] Elias C, Saper CB, Maratos-Flier E, *et al.* Chemically defined projections linking the mediobasal hypothalamus and the lateral hypothalamic area. J Comp Neurol 1998; 402: 442-59.

[16] Broberger C, Johansen J, Brismar H, *et al.* Changes in neuropeptide Y receptors and pro-opiomelanocortin in the anorexia (anx/anx) mouse hypothalamus. J Neurosci 1999; 19: 7130-9.

[17] Vrang N, Kristensen P, Tang-Christensen M, Larsen PJ. Effects of leptin on arcuate pro-opiomelanocortin and cocaine-amphetamine-regulated transcript expression are independent of circulating levels of corticosterone. J Neuroendocrinol 2002; 14: 880-6.

[18] Elias CF, Lee CE, Kelly JF, *et al.* Characterization of CART neurons in the rat and human hypothalamus. J Comp Neurol 2001; 432: 1-19.

[19] Swanson LW, Kuypers HG. A direct projection from the ventromedial nucleus and retrochiasmatic area of the hypothalamus to the medulla and spinal cord of the rat. Neurosci Lett 1980; 17: 307-12.

[20] Baker RA, Herkenham M. Arcuate nucleus neurons that project to the hypothalamic paraventricular nucleus: neuropeptidergic identity and consequences of adrenalectomy on mRNA levels in the rat. J Comp Neurol 1995; 358: 518-30.

[21] Elias CF, Aschkenasi C, Lee C, *et al.* Leptin differentially regulates NPY and POMC neurons projecting to the lateral hypothalamic area. Neuron 1999; 23: 775-86.

[22] Elias CF, Lee C, Kelly J, *et al.* Leptin activates hypothalamic CART neurons projecting to the spinal cord. Neuron 1998; 21: 1375-85.

[23] Johnson RF, Morin LP, Moore RY. Retinohypothalamic projections in the hamster and rat demonstrated using cholera toxin. Brain Res 1988; 462: 301-12.

[24] Vrang N, Mrosovsky N, Mikkelsen JD. Afferent projections to the hamster intergeniculate leaflet demonstrated by retrograde and anterograde tracing. Brain Res Bull 2003; 59: 267-88.

[25] Zheng H, Patterson LM, Phifer CB, Berthoud HR. Brain stem melanocortinergic modulation of meal size and identification of

hypothalamic POMC projections. Am J Physiol Regul Integr Comp Physiol 2005; 289: R247-58.

[26] Sim LJ, Joseph SA. Arcuate nucleus projections to brainstem regions which modulate nociception. J Chem Neuroanat 1991; 4: 97-109.

[27] Li C, Chen P, Smith MS. Morphological evidence for direct interaction between arcuate nucleus neuropeptide Y (NPY) neurons and gonadotropin-releasing hormone neurons and the possible involvement of NPY Y1 receptors. Endocrinology 1999; 140: 5382-90.

[28] Collin M, Backberg M, Ovesjo ML, *et al.* Plasma membrane and vesicular glutamate transporter mRNAs/proteins in hypothalamic neurons that regulate body weight. Eur J Neurosci 2003; 18: 1265-78.

[29] Hentges ST, Nishiyama M, Overstreet LS, *et al*. GABA release from proopiomelanocortin neurons. J Neurosci 2004; 24: 1578-83.

[30] Backberg M, Madjid N, Ogren SO, Meister B. Down-regulated expression of agouti-related protein (AGRP) mRNA in the hypothalamic arcuate nucleus of hyperphagic and obese tub/tub mice. Brain Res Mol Brain Res 2004; 125: 129-39.

[31] Williams K, Margatho LO, Lee CE, *et al.* Marked segregation of acute leptin and insulin effects in distinct populations of arcuate POMC neurons. J Neurosci 2009; (in press).

[32] 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.

[33] Rouille Y, Duguay SJ, Lund K, *et al.* Proteolytic processing mechanisms in the biosynthesis of neuroendocrine peptides: the subtilisin-like proprotein convertases. Front Neuroendocrinol 1995; 16: 322-61.

[34] del GEM, Santoro N, Cirillo G, *et al.* 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.

[35] Yanik T, Dominguez G, Kuhar MJ, Del GEM, Loh YP. The Leu34Phe ProCART mutation leads to cocaine- and amphetamine-regulated transcript (CART) deficiency: a possible cause for obesity in humans. Endocrinology 2006; 147: 39-43.

[36] Yamada K, Yuan X, Otabe S, *et al.* Sequencing of the putative promoter region of the cocaine- and amphetamine-regulated-transcript gene and identification of polymorphic sites associated with obesity. Int J Obes Relat Metab Disord 2002; 26: 132-6.

[37] Guerardel A, Barat-Houari M, Vasseur F, *et al.* Analysis of sequence variability in the CART gene in relation to obesity in a Caucasian population. BMC Genet 2005; 6: 19.

[38] Vettor R, Fabris R, Pagano C, Federspil G. Neuroendocrine regulation of eating behavior. J Endocrinol Invest 2002; 25: 836-54.

[39] Qing K, Chen Y. Central CART gene delivery by recombinant AAV vector attenuates body weight gain in diet-induced-obese rats. Regul Pept 2007; 140: 21-6.

[40] Kokkotou E, Jeon JY, Wang X, *et al.* Mice with MCH ablation resist diet-induced obesity through strain-specific mechanisms. Am J Physiol Regul Integr Comp Physiol 2005; 289: R117-24.

[41] Wang ZW, Zhou YT, Kakuma T, *et al.* Comparing the hypothalamic and extrahypothalamic actions of endogenous hyperleptinemia. Proc Natl Acad Sci USA 199; 9 96: 10373-8.

[42] Germano CM, de CM, Rorato R, *et al.* Time course effects of adrenalectomy and food intake on cocaine- and amphetamine-regulated transcript expression in the hypothalamus. Brain Res 2007; 1166: 55-64.

[43] Dominguez G. The CART gene: structure and regulation. Peptides 2006; 27: 1913-8.

[44] Dominguez G, Lakatos A, Kuhar MJ. Characterization of the cocaine- and amphetamine-regulated transcript (CART) peptide gene promoter and its activation by a cyclic AMP-dependent signaling pathway in GH3 cells. J Neurochem 2002; 80: 885-93.

[45] Dominguez G, Kuhar MJ. Transcriptional regulation of the CART promoter in CATH α cells. Brain Res Mol Brain Res 2004; 126: 22-9.

[46] Barrett P, Davidson J, Morgan P. CART gene promoter transcription is regulated by a cyclic adenosine monophosphate response element. Obes Res 2002; 10: 1291-8.

[47] Barrett P, Morris MA, Moar KM, *et al.* The differential regulation of CART gene expression in a pituitary cell line and primary cell cultures of ovine pars tuberalis cells. J. Neuroendocrinol 2001; 13: 347-52.

[48] Ahn S, Olive M, Aggarwal S, *et al*. A dominant-negative inhibitor of CREB reveals that it is a general mediator of stimulus-dependent transcription of c-fos. Mol Cell Biol 1998; 18: 967-77.

[49] Bannister AJ, Oehler T, Wilhelm D, Angel P, Kouzarides T. Stimulation of c-Jun activity by CBP: c-Jun residues Ser63/73 are required for CBP induced stimulation *in vivo* and CBP binding *in vitro*. Oncogene 1995; 11: 2509-14.

[50] Goldman PS, Tran VK, Goodman RH. The multifunctional role of the co-activator CBP in transcriptional regulation. Recent Prog Horm Res 1997; 52: 103-19; discussion 119-20.

[51] Hai T, Curran T. Cross-family dimerization of transcription factors Fos/Jun and ATF/CREB alters DNA binding specificity. Proc Natl Acad Sci USA 1991; 88: 3720-4.

[52] Hu PP, Harvat BL, Hook SS, *et al*. c-Jun enhancement of cyclic adenosine 3',5'-monophosphate response element-dependent transcription induced by transforming growth factor-beta is independent of c-Jun binding to DNA. Mol Endocrinol 1999; 13: 2039-48.

[53] Jin K, Mao XO, Simon RP, Greenberg DA. Cyclic AMP response element binding protein (CREB) and CREB binding protein (CBP) in global cerebral ischemia. J Mol Neurosci 2001; 16: 49-56.

[54] Powell JD, Lerner CG, Ewoldt GR, Schwartz RH. The -180 site of the IL-2 promoter is the target of CREB/CREM binding in T cell anergy. J Immunol 1999; 163: 6631-9.

[55] Shenkar R, Yum HK, Arcaroli J, Kupfner J, Abraham E. Interactions between CBP, NF-kappaB, and CREB in the lungs after hemorrhage and endotoxemia. Am J Physiol Lung Cell Mol Physiol 2001; 281: L418-26.

[56] Jones DC, Kuhar MJ. Cocaine-amphetamine-regulated transcript expression in the rat nucleus accumbens is regulated by adenylyl cyclase and the cyclic adenosine 5'-monophosphate/protein kinase a second messenger system. J Pharmacol Exp Ther 2006; 317: 454-61.

[57] Dantzer R. Cytokine-induced sickness behavior: mechanisms and implications. Ann N Y Acad Sci 2001; 933: 222-34.

[58] Sergeyev V, Broberger C, Hokfelt T. Effect of LPS administration on the expression of POMC, NPY, galanin, CART and MCH mRNAs in the rat hypothalamus. Brain Res Mol Brain Res 2001; 90: 93-100.

[59] Voss JW, Rosenfeld MG. Anterior pituitary development: short tales from dwarf mice. Cell 1992; 70: 527-30.

[60] Hunter RG, Vicentic A, Rogge G, Kuhar MJ. The effects of cocaine on CART expression in the rat nucleus accumbens: a possible role for corticosterone. Eur J Pharmacol 2005; 517: 45-50.

[61] Balkan B, Koylu E, Pogun S, Kuhar MJ. Effects of adrenalectomy on CART expression in the rat arcuate nucleus. Synapse 2003; 50: 14-9.

[62] Vrang N, Larsen PJ, Tang-Christensen M, Larsen LK, Kristensen P. Hypothalamic cocaine-amphetamine regulated transcript (CART) is regulated by glucocorticoids. Brain Res 2003; 965: 45-50.

[63] Pritchard LE, Turnbull AV, White A. Pro-opiomelanocortin processing in the hypothalamus: impact on melanocortin signalling and obesity. J Endocrinol 2002; 172: 411-21.

[64] Challis BG, Coll AP, Yeo GS, *et al.* Mice lacking pro-opiomelanocortin are sensitive to high-fat feeding but respond normally to the acute anorectic effects of peptide-YY(3-36). Proc Natl Acad Sci USA 2004; 101: 4695-700.

[65] Barnea A, Cho G, Porter JC. A reduction in the concentration of immunoreactive corticotropin, melanotropin and lipotropin in the brain of the aging rat. Brain Res 1982; 232: 345-53.

[66] Gramsch C, Kleber G, Hollt V, *et al.* Pro-opiocortin fragments in human and rat brain: beta-endorphin and alpha-MSH are the predominant peptides. Brain Res 1980; 192: 109-19.

[67] Florijn WJ, Mulder AH, Versteeg DH, Gispen WH. Adrenocorticotropin/alpha-melanocyte-stimulating hormone (ACTH/MSH)-like peptides modulate adenylate cyclase activity in rat brain slices: evidence for an ACTH/MSH receptor-coupled mechanism. J Neurochem 1993 60: 2204-11.

[68] Emeson RB, Eipper BA. Characterization of pro-ACTH/endorphin-derived peptides in rat hypothalamus. J Neurosci 1986; 6: 837-49.

[69] Ellacott KL, Cone RD. The central melanocortin system and the integration of short- and long-term regulators of energy homeostasis. Recent Prog Horm Res 2004; 59: 395-408.

[70] Tung YC, Piper SJ, Yeung D, O'Rahilly S, Coll AP. A comparative study of the central effects of specific proopiomelancortin (POMC)-derived melanocortin peptides on food intake and body weight in pomc null mice. Endocrinology 2006; 147: 5940-7.

[71] Jackson RS, Creemers JW, Ohagi S, *et al.* Obesity and impaired prohormone processing associated with mutations in the human prohormone convertase 1 gene. Nat Genet 1997; 16: 303-6.

[72] Jackson RS, Creemers JW, Farooqi IS, *et al.* Small-intestinal dysfunction accompanies the complex endocrinopathy of human proprotein convertase 1 deficiency. J Clin Invest 2003; 112: 1550-60.

[73] Lloyd DJ, Bohan S, Gekakis N. Obesity, hyperphagia and increased metabolic efficiency in Pc1 mutant mice. Hum Mol Genet 2006; 15: 1884-93.

[74] Lee YS, Challis BG, Thompson DA, *et al.* A POMC variant implicates beta-melanocyte-stimulating hormone in the control of human energy balance. Cell Metab 2006; 3: 135-40.

[75] Ibrahim N, Bosch MA, Smart JL, *et al.* Hypothalamic proopiomelanocortin neurons are glucose responsive and express K(ATP) channels. Endocrinology 2003; 144: 1331-40.

[76] Kelly MJ, Loose MD, Ronnekleiv OK. Opioids hyperpolarize beta-endorphin neurons via mu-receptor activation of a potassium conductance. Neuroendocrinology 1990 52: 268-75.

[77] Grandison L, Guidotti A. Stimulation of food intake by muscimol and beta endorphin. Neuropharmacology 1977; 16: 533-6.

[78] Kalra SP, Horvath TL. Neuroendocrine interactions between galanin, opioids, and neuropeptide Y in the control of reproduction and appetite. Ann N Y Acad Sci 1998; 863: 236-40.

[79] Olszewski PK, Wirth MM, Grace MK, Levine AS, Giraudo SQ. Evidence of interactions between melanocortin and opioid systems in regulation of feeding. Neuroreport 2001; 12: 1727-30.

[80] Mizuno TM, Kelley KA, Pasinetti GM, Roberts JL, Mobbs CV. Transgenic neuronal expression of proopiomelanocortin attenuates hyperphagic response to fasting and reverses metabolic impairments in leptin-deficient obese mice. Diabetes 2003; 52: 2675-83.

[81] Schwartz Mw, Baskin DG, Bukowski TR, *et al.* Specificity of leptin action on elevated blood glucose levels and hypothalamic neuropeptide Y gene expression in ob/ob mice. Diabetes 1996; 45: 531-5.

[82] Morrison CD, Morton GJ, Niswender KD, Gelling RW, Schwartz MW. Leptin inhibits hypothalamic Npy and Agrp gene expression *via* a mechanism that requires phosphatidylinositol 3-OH-kinase signaling. Am J Physiol Endocrinol Metab 2005; 289: E1051-7.

[83] Kitamura T, Feng Y, Kitamura YI, *et al.* Forkhead protein FoxO1 mediates Agrp-dependent effects of leptin on food intake. Nat Med 2006; 12: 534-40.

[84] Young JI, Otero V, Cerdan MG, *et al.* Authentic cell-specific and developmentally regulated expression of pro-opiomelanocortin genomic fragments in hypothalamic and hindbrain neurons of transgenic mice. J Neurosci 1998; 18: 6631-40.

[85] Sanchez VC, Goldstein J, Stuart RC, *et al.* Regulation of hypothalamic prohormone convertases 1 and 2 and effects on processing of prothyrotropin-releasing hormone. J Clin Invest 2004; 114: 357-69.

[86] Kalra SP, Dube MG, Pu S, *et al*. Interacting appetite-regulating pathways in the hypothalamic regulation of body weight. Endocr Rev 1999 20: 68-100.

[87] Nillni EA. Regulation of prohormone convertases in hypothalamic neurons: implications for prothyrotropin-releasing hormone and proopiomelanocortin. Endocrinology 2007; 148: 4191-200.

[88] Perello M, Stuart RC, Nillni EA. Differential effects of fasting and leptin on proopiomelanocortin peptides in the arcuate nucleus and in the nucleus of the solitary tract. Am J Physiol Endocrinol Metab 2007; 292: E1348-57.

[89] Cheung C, Clifton DK, Steiner RA. Proopiomelanocortin neurons are direct targets for leptin in the hypothalamus. Endocrinology 1997 138: 4489-92.

[90] Thornton J, Cheung CC, Clifton DK, Steiner RA. Regulation of hypothalamic proopiomelanocortin mRNA by leptin in ob/ob mice. Endocrinology 1997 138: 5063-6.

[91] Ftouhi N, Day R, Mbikay M, Chretien M, Seidah NG. Gene organization of the mouse pro-hormone and pro-protein convertase PC1. DNA Cell Biol 1994; 13: 395-407.

[92] Fox DL, Vella KR, Good DJ. Energy balance pathways converging on the Nhlh2 transcription factor. Front Biosci 2007; 12: 3983-93.

[93] Bruning JC, Gautam D, Burks DJ, *et al.* Role of brain insulin receptor in control of body weight and reproduction. Science 2000 289: 2122-5.

[94] Burks DJ, de Mora JF, Schubert M, *et al.* IRS-2 pathways integrate female reproduction and energy homeostasis. Nature 2000; 407: 377-82.

[95] Niswender KD, Morrison CD, Clegg DJ, *et al*. Insulin activation of phosphatidylinositol 3-kinase in the hypothalamic arcuate nucleus: a key mediator of insulin-induced anorexia. Diabetes 2003; 52: 227-31.

[96] Niswender KD, Morton GJ, Stearns WH, *et al.* Intracellular signalling. Key enzyme in leptin-induced anorexia. Nature 2001; 413: 794-5.

[97] Tang ED, Nunez G, Barr FG, Guan KL. Negative regulation of the forkhead transcription factor FKHR by Akt. J Biol Chem 1999; 274: 16741-6.

[98] Matsuzaki H, Daitoku H, Hatta M, Tanaka K, Fukamizu A. Insulin-induced phosphorylation of FKHR (Foxo1) targets to proteasomal degradation. Proc Natl Acad Sci USA 2003; 100: 11285-90.

[99] Kim M, Pak YK, Jang P-G, *et al.* Role of hypothalamic Foxo1 in the regulation of food intake and energy homeostasis. Nat Neurosci 2006 9: 901-6.

[100] Munzberg H, Huo L, Nillni EA, Hollenberg AN, Bjorbaek C. Role of signal transducer and activator of transcription 3 in regulation of hypothalamic proopiomelanocortin gene expression by leptin. Endocrinology 2003; 144: 2121-31.

[101] Good DJ, Porter FD, Mahon KA, *et al.* Hypogonadism and obesity in mice with a targeted deletion of the Nhlh2 gene. Nat Genet 1997; 15: 397-401.

[102] Jing E, Nillni EA, Sanchez VC, Stuart RC, Good DJ. Deletion of the Nhlh2 transcription factor decreases the levels of the anorexigenic peptides alpha melanocyte-stimulating hormone and thyrotropin-releasing hormone and implicates prohormone convertases I and II in obesity. Endocrinology 2004; 145: 1503-13.

[103] Fox DL, Good DJ. Nescient helix-loop-helix 2 interacts with signal transducer and activator of transcription 3 to regulate transcription of prohormone convertase 1/3. Mol Endocrinol 2008; 22: 1438-48.

[104] Blum M, Roberts JL, Wardlaw SL. Androgen regulation of proopiomelanocortin gene expression and peptide content in the basal hypothalamus. Endocrinology 1989; 124: 2283-8.

[105] Treiser SL, Wardlaw SL. Estradiol regulation of proopiomelanocortin gene expression and peptide content in the hypothalamus. Neuroendocrinology 1992; 55: 167-73.

[106] Pelletier G, Li S, Luu-The V, Labrie F. Oestrogenic regulation of pro-opiomelanocortin, neuropeptide Y and corticotrophin-releasing hormone mRNAs in mouse hypothalamus. J Neuroendocrinol 2007; 19: 426-31.

[107] Gao Q, Mezei G, Nie Y, *et al*. Anorectic estrogen mimics leptin's effect on the rewiring of melanocortin cells and Stat3 signaling in obese animals. Nat Med 2007; 13: 89-94.

[108] Hirosawa M, Minata M, Harada KH, *et al.* Ablation of estrogen receptor alpha (ERalpha) prevents upregulation of POMC by leptin and insulin. Biochem Biophys Res Commun 2008; 371: 320-3.

[109] Catalano S, Mauro L, Marsico S, *et al*. Leptin induces, via ERK1/ERK2 signal, functional activation of estrogen receptor alpha in MCF-7 cells. J Biol Chem 2004; 279: 19908-15.

[110] Xu A, Marie L, Kaelin CB, Barsh GS. Inactivation of signal transducer and activator of transcription 3 in proopiomelanocortin (Pomc) neurons causes decreased pomc expression, mild obesity, and defects in compensatory refeeding. Endocrinology 2007; 148: 72-80.

[111] Malyala A, Kelly MJ, Ronnekleiv OK. Estrogen modulation of hypothalamic neurons: activation of multiple signaling pathways and gene expression changes. Steroids 2005; 70: 397-406.

[112] Clegg D, Brown LM, Woods SC, Benoit SC. Gonadal hormones determine sensitivity to central leptin and insulin. Diabetes 2006; 55: 978-87.

[113] Ste Marie L, Miura GI, Marsh DJ, Yagaloff K, Palmiter RD. A metabolic defect promotes obesity in mice lacking melanocortin-4 receptors. Proc Natl Acad Sci USA 2000; 97: 12339-44.

[114] Kiss J, Leranth C, Halasz B. Serotoninergic endings on VIP-neurons in the suprachiasmatic nucleus and on ACTH-neurons in the arcuate nucleus of the rat hypothalamus: a combination of high resolution autoradiography and electron microscopic immunocytochemistry. Neurosci Lett 1984 44: 119-24.

[115] Heisler LK, Cowley MA, Tecott LH, *et al.* Activation of central melanocortin pathways by fenfluramine. Science 2002; 297: 609-11.

[116] Xu Y, Jones JE, Kohno D, *et al.* 5-HT2CRs expressed by pro-opiomelanocortin neurons regulate energy homeostasis. Neuron 2008; 60: 582-9.

[117] Zhou L, Sutton GM, Rochford JJ, *et al.* Serotonin 2C receptor agonists improve type 2 diabetes *via* melanocortin-4 receptor signaling pathways. Cell Metab 2007; 6: 398-405.

[118] Lam DD, Przydzial MJ, Ridley SH, *et al.* Serotonin 5-HT2C receptor agonist promotes hypophagia via downstream activation of melanocortin 4 receptors. Endocrinology 2008; 149: 1323-8.

[119] Newell-Price J. Proopiomelanocortin gene expression and DNA methylation: implications for Cushing's syndrome and beyond. J Endocrinol 2003; 177: 365-72.

Received: June 08, 2009 Revised: August 04, 2009 Accepted: August 04, 2009