

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

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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 through alternative pathways such as IRS- phosphoinositide 3-kinase (PI3K) signaling [8-10].

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

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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 anti-

bodies 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-adrenocorticotrophic 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.

Melanocortins 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 β -MSH, γ -LPH, and γ_1 - and γ_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 (ER α) [107, 108]. ER α 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 ER α 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-HT_{2C} receptors, with co-expression being greatest in the caudal ARC [115]. 5-HT_{2C} 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-HT_{2C} 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 to be a critical area of investigation with important implications for the treatment of obesity in humans.

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