Mol Neurobiol (2012) 45:225–233
DOI 10.1007/s12035-012-8240-6

Functional Heterogeneity of Arcuate Nucleus Pro-Opiomelanocortin Neurons: Implications for Diverging Melanocortin Pathways

Jong-Woo Sohn · Kevin W. Williams

Received: 12 September 2011 / Accepted: 26 January 2012 / Published online: 12 February 2012

Abstract Arcuate nucleus (ARC) pro-opiomelanocortin (POMC) neurons are essential regulators of food intake, energy expenditure, and glucose homeostasis. POMC neurons integrate several key metabolic signals that include neurotransmitters and hormones. The change in activity of POMC neurons is relayed to melanocortin receptors in distinct regions of the central nervous system. This review will summarize the role of leptin and serotonin receptors in regulating the activity of POMC neurons and provide a model in which different melanocortin pathways regulate energy and glucose homeostasis.

Keywords Melanocortin · Leptin · Serotonin · Segregation · Insulin · Energy expenditure · Body weight · Adiposity · Hyperglycemia · TRPC · PI3K

Introduction

The prevalence of obesity and type 2 diabetes poses an enormous health risk worldwide. A recent report demonstrated that the incidences of both diseases have experienced rapid growth over the last two decades [1]. Not surprisingly, in order to treat diseases like obesity, diabetes, and associated comorbidities, researchers have been developing novel strategies to better understand the underlying mechanisms regulating energy and glucose metabolism. Recent insights have focused on the identification of the circuits within the central nervous system that are responsible for regulating energy and glucose homeostasis. The adipose-derived peptide leptin and the neurotransmitter serotonin both have profound effects on energy and glucose homeostasis. The current review summarizes recent findings on distinct roles of leptin and serotonin in a subset of neurons located in the arcuate nucleus of the mediobasal hypothalamus called pro-opiomelanocortin (POMC) neurons. This review will also outline a model of a distributed cellular network for leptin, serotonin, and melanocortin receptors within the central nervous system which may contribute to the regulation of glucose and energy homeostasis.

J.-W. Sohn · K. W. Williams (✉)
Division of Hypothalamic Research,
Department of Internal Medicine,
University of Texas Southwestern Medical Center at Dallas,
5323 Harry Hines Boulevard,
Dallas, TX 75390-9077, USA
e-mail: kevin.williams@utsouthwestern.edu

The Arcuate Nucleus: a Key Site for the Regulation of Energy and Glucose Homeostasis

The mediobasal hypothalamus is critical in regulating food intake and body weight [2–4]. The arcuate nucleus of the hypothalamus (ARC), which includes the POMC and agouti-related peptide (AgRP) neurons, is arguably the most-studied brain region as it relates to the neural control of food intake and body weight. POMC is a gene that encodes a precursor polypeptide which undergoes posttranslational processing giving rise to several peptides called melanocortins. The melanocortins are involved in a variety of biological activities. For example, the melanocortin neuropeptide α-melanocyte-stimulating hormone (α-MSH) is an melanocortin 3 and 4 receptor (MC3R and MC4R) agonist which along with its analogs has been shown to potently inhibit food intake via activation of melanocortin neurons within the central nervous system [5–8]. Neurons expressing

AgRP, the inverse agonist (functional antagonist) of melanocortin receptors, are located adjacent to ARC POMC neurons. Importantly, AgRP neurons exclusively colocalize with neuropeptide Y (NPY) in the arcuate nucleus [9–12]. NPY has been associated with several biological activities including learning and memory, nociception, and epilepsy [13,14]; however, NPY has a primary role of stimulating food intake and regulating energy expenditure [15]. Not surprisingly, both POMC and NPY/AgRP neurons integrate various hormones and neurotransmitters, such as leptin, insulin, and serotonin, which are important in the regulation of energy and glucose homeostasis (Fig. 1a) and reviewed here [16,17]. Thus, both α-MSH and NPY/AgRP serve as potent modulators of food intake and body weight.

Prior work demonstrated via the use of conventional knockout models that loss of POMC or the MC3Rs and MC4Rs resulted in hyperphagia and obesity [18–21]. Interestingly, mice deficient for AgRP and/or NPY demonstrated normal food intake, body weight, and adiposity [22]. Similarly, toxin-induced ablation of AgRP/NPY neurons early in development resulted in modest effects on food intake [23,24]. However, toxin-induced ablation of AgRP neurons in adults resulted in a rapid suppression of food intake [23]. A parallel study also demonstrated that in adult mice ablation of POMC neurons or NPY/AgRP neurons resulted in acutely increased or decreased food intake, respectively [25]. These data suggested that although developmental loss of the peptides NPY/AgRP or the NPY/AgRP neurons entirely may lead to developmental mechanisms compensating for the alterations in food intake, mature POMC and NPY/AgRP neurons are important in the regulation of food intake and energy homeostasis. Two studies recently extended these observations and reinforced an acute role for POMC and NPY/AgRP neurons in the regulation of feeding behavior. Aponte et al. [26] used optogenetics to demonstrate that stimulation of ARC POMC neurons suppressed feeding, while stimulating AgRP neurons increased food intake in adult mice. The authors also found that the POMC-induced suppression of food intake required melanocortin signaling; however, the AgRP-induced hyperphagia was independent of melanocortin activity. These data importantly demonstrated that acute stimulation of ARC POMC or AgRP neurons directly regulates food intake. Moreover, these data highlight an important role for other signals released by AgRP neurons in feeding behavior. A parallel study by Krashes and colleagues used designer receptors exclusively activated by designer drugs (DREADD) in order to assess the role of AgRP neurons in energy balance and feeding behavior [27]. Stimulation of AgRP neurons profoundly increased food intake and decreased energy expenditure. Not surprisingly, these deficits in energy balance ultimately led to increased body weight and adiposity. Importantly, the elevated food intake and body weight could be normalized by removal of the DREADD activator. Conversely, inhibition of AgRP cellular activity in hungry mice resulted in a reduction of food intake. Notably, stimulating AgRP neurons of animals fed an ad libitum diet failed to influence locomotor activity; however, stimulation of AgRP neurons in the absence of food resulted in a striking increase of locomotor activity. Together these data highlight an important role for POMC and NPY/AgRP neurons in the regulation of food intake and further suggest that NPY/AgRP neurons regulate motivations associated with food-seeking behaviors.

In addition to regulating energy balance, feeding behavior, and locomotor activity; recent evidence has shown that modulation of POMC and NPY/AgRP local circuits in the mediobasal hypothalamus may also contribute to glucose balance [28–32]. However it has been unclear whether this regulation is acute or depends more on genomic changes and whether the activity of POMC neurons alone or NPY/AgRP neurons also influences peripheral glucose production/reuptake. Similarly, POMC and NPY/AgRP neurons have been implicated in the physiology of addiction and reward; however, exactly how and to what extent these neurons influence these behaviors is unclear [17,33,34]. Thus these studies provide a framework for several exciting areas of future investigation and will undoubtedly extend our current understanding on the various roles of the melanocortin circuit.

Intrinsic and Extrinsic Regulation of ARC POMC and NPY/AgRP Neurons by Leptin

The adipocyte-derived hormone leptin and its receptors (LepRs) ameliorate hyperphagia and obesity [35–37]. Mice which lack leptin (*ob*/*ob*) or LepRs (*db*/*db*) are markedly hyperphagic and obese as well as associated with other abnormalities including diabetes, infertility, impaired growth, high bone mass, and hypercorticosteronemia [37–41]. Interestingly, intracerebroventricular injections of leptin recapitulated the effect of peripheral leptin injections to reduce food intake and body weight [35,42,43]. In addition, the obese phenotype observed in mice lacking LepRs in neurons mirrored the obesity observed in *db/db* mice [44]. Together these data suggested that most of leptin's effects are mediated by LepRs within central nervous system [45].

Subsequent studies attempted to identify the specific brain regions mediating leptin effects. Given the role of ARC POMC neurons in regulating feeding behavior and body weight, the requirement of LepRs in POMC neurons was first tested using the cyclization recombination locus of X over P1 technology to delete LepRs only in POMC neurons [46]. Mice lacking LepRs specifically in POMC neurons were obese; however, the obesity was significantly less than that observed in *db/db* mice. This modest obesity

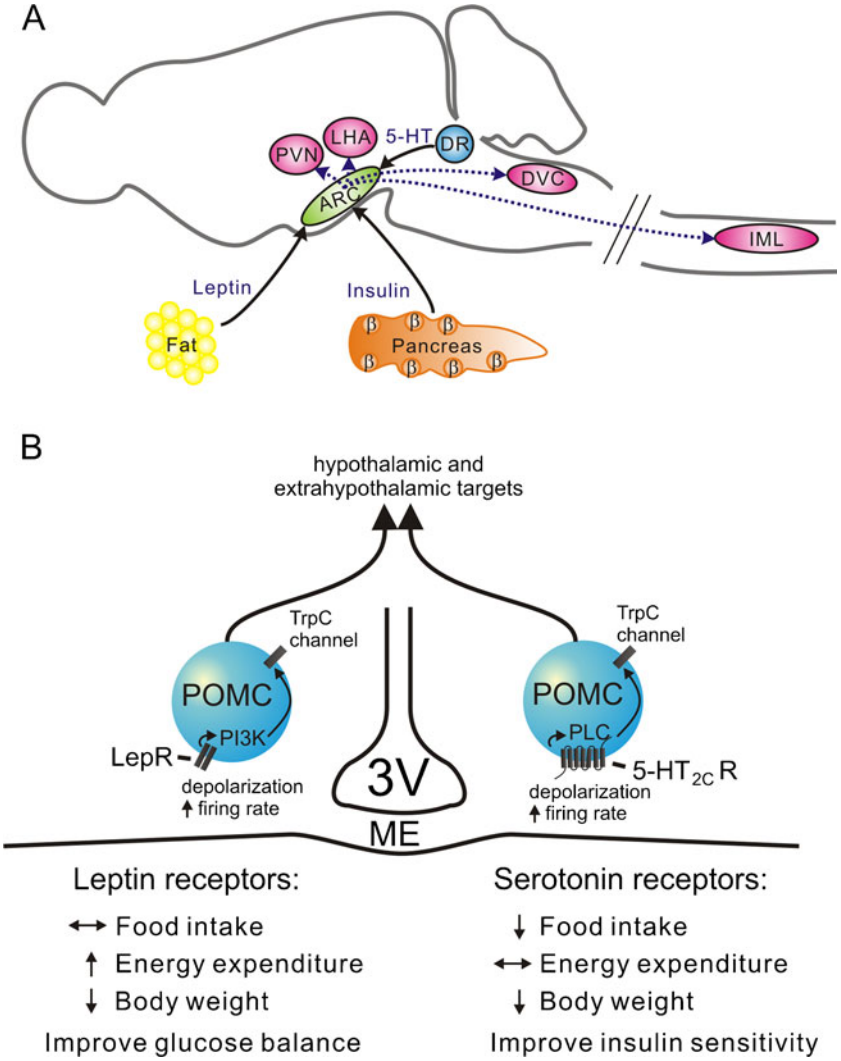

Fig. 1 The arcuate nucleus integrates a variety of metabolic and neuronal signals which are relayed within the central nervous system to regulate energy and glucose homeostasis. **a** Leptin and insulin are peripheral hormones which influence energy and glucose homeostasis via actions within the arcuate nucleus. The neurotransmitter serotonin also influences the activity of neurons within the arcuate nucleus. These signals ultimately influence distinct central nuclei downstream of the arcuate nucleus which also express melanocortin receptors. Importantly, many of these downstream targets have recently been demonstrated to be direct targets of these same metabolic and neuronal signals, thus establishing a distributed network of neurons responsible for energy and glucose balance. *ARC* arcuate nucleus, *DR* dorsal raphe nucleus, *DVC* dorsal vagal complex, *IML* intermediolateral column of spinal cord, *LHA* lateral hypothalamic nucleus, *PVN* paraventricular hypothalamic nucleus, *β* pancreatic β-cell. **b** Leptin receptors (*LepRs*) on POMC neurons have been shown to increase energy expenditure resulting in a reduction of body weight independent of changes in food intake. Moreover, LepRs on POMC neurons are required for glucose regulation. Opposite to the effects of LepRs on POMC neurons, serotonin 2 C receptors (5-HT_{2C}Rs) decrease food intake resulting in a reduction in body weight independent of changes in energy expenditure. Selective reexpression of 5-HT_{2C}Rs in POMC neurons also restores hepatic insulin resistance found in mice deficient of 5-HT_{2C}Rs

resulted from decreased energy expenditure leading to increased fat mass, while surprisingly hyperphagia was not observed in these mice [47]. Notably, deletions of LepRs in SF-1 neurons of the ventromedial hypothalamic nucleus (VMH) also led to obesity, and the obesity was additive when LepRs were deleted from both POMC and SF-1 neurons [48,49]. Moreover, deficiency of LepRs in AgRP neurons resulted in decreased locomotor activity and modest obesity [50]. Again, concomitant deletion of LepRs in POMC and AgRP neurons lead to an additive effect on body weight and unmasks a striking hyperphagic phenotype. Interestingly, leptin partially reduced food intake in mice deficient for LepRs in AgRP and POMC neurons, supporting a role for leptin to regulate food intake outside of the ARC POMC and NPY/AgRP neurons [50]. These results suggested that the central effects of leptin require ARC POMC neurons; however, LepRs are also required in multiple nuclei, including but not limited to ARC NPY/AgRP and VMH SF1 neurons, for the regulation of glucose and energy balance [17]. A more recent study examined the

requirement of LepRs in the nucleus tractus solitarius (NTS) of the caudal medulla. Mice deficient for LepRs within the NTS exhibited an increased rate of weight change over time and hyperphagia [51]. These data are in agreement with a separate study in which LepRs were knocked down in the rat hindbrain resulting in increased body weight [52]. Collectively, these data support a role for LepRs in the NTS to regulate body weight and feeding behavior. Thus, although POMC and NPY/AgRP neurons are important contributors to the central effects of leptin, these recent data support a distributed network of neurons which contribute to the various beneficial effects of leptin.

The cellular mechanisms and network properties of leptin action on POMC neurons has been intensely studied by many groups. Leptin has been shown to activate multiple signaling cascades resulting in acute changes of neuronal activity as well as long-term genomic regulation ultimately contributing to different facets of energy and glucose balance [17]. Initial reports suggested that leptin acutely hyperpolarized an unidentified neuronal population in the mediobasal hypothalamus [53,54]. Subsequent studies suggested that leptin hyperpolarized NPY/AgRP neurons in the ARC while depolarizing ARC POMC neurons and VMH SF1 neurons [48,55–58]. Upon further examination, the leptin-induced depolarization of ARC POMC neurons was shown to be concomitant with the activation of a nonselective cation conductance which was more recently identified as a transient receptor potential C (TRPC) channel [57–59]. In addition, the inhibition of adjacent NPY/AgRP neurons by leptin suppressed GABAergic inputs to ARC POMC neurons which may provide an indirect activation of ARC POMC neurons [58,60]. Importantly, leptin failed to depolarize ARC POMC neurons which were deficient for phosphatitylinositol-3-kinase (PI3K) regulatory subunits, p85α and p85β (Fig. 1b). Moreover, energy balance in response to high fat diet was not affected by the deficiency of both p85α and p85β regulatory subunits [57]. These results suggested that PI3K in POMC neurons exclusively mediate the acute anorexigenic effects of leptin, while the long-term effects of leptin to regulate energy expenditure and glucose balance may be mediated by the other prevalent signaling pathways [57,61–63]. However, a recent report suggested that the segregation of such pathways may not be as clear cut. A separate group recently demonstrated that the catalytic PI3K subunit, p110β, but not p110α is required for the leptin-induced depolarization of ARC POMC neurons [61]. Moreover, the anorexigenic effect of leptin was absent in POMC p110β null mice. Interestingly, selective deletion of p110β from POMC cells resulted in central leptin resistance, increased adiposity, and diet-induced obesity, while POMC p110α null mice experienced minor energy expenditure deficits except when exposed to HFD. Together these data suggest that there may be an underappreciated role of PI3K signaling in the regulation of energy homeostasis in ARC POMC neurons.

Recent work has also suggested that indirect regulation of POMC neurons may play a far larger role in the regulation of energy balance than previously expected. Specifically, NPY/AgRP colocalize with GABA in synaptic boutons which synapse onto ARC POMC neurons, supporting a model in which NPY/AgRP neuronal activity may provide an indirect regulation of ARC POMC neurons [58,60,64]. In addition to NPY/AgRP/GABA neurons, glutamatergic neurons in the adjacent VMH have been shown to project to POMC neurons [65], adding an additional level of complexity to the indirect regulation of POMC neurons via hypothalamic local circuitry. Importantly, Vong et al. [66] recently demonstrated that leptin action on non-AgRP GABAergic neurons prevents obesity to a far greater extent than leptin action on glutamatergic neurons. Moreover, the authors demonstrated that leptin reduces the inhibitory tone on ARC POMC neurons, and this disinhibition probably mediates, at least in part, leptin's antiobesity effects. Thus, understanding the regulation of POMC neurons has been greatly facilitated by the recruitment of genetically modified mouse models. Moreover, evidence from recent studies demonstrated that many aspects of energy and glucose homeostasis are regulated by POMC neurons but importantly other chemically identified neurons in the central nervous system also contribute to the leptin-induced regulation of energy and glucose balance.

ARC POMC and NPY/AgRP Neurons: Important Cellular Targets of Serotonin

Serotonin (5-hydroxytryptamine; 5-HT) regulates energy and glucose homeostasis via actions in the central nervous system [67,68]. Increased release and/or reduced reuptake of serotonin have been shown to underlie the antiobesity effects of several serotonergic pharmacological agents [69,70]. Specifically, fenfluramine (d-FEN) was a very effective and successful antiobesity drug before it was removed from the market due to serious cardiopulmonary side effects including valvular heart diseases and pulmonary hypertension [71]. Recent studies have suggested that the antiobesity effects of serotonin and serotonergic agents like d-FEN require serotonin 2C receptors (5-HT_{2C}Rs). For example, global deletion of 5-HT_{2C}Rs led to hyperphagia and obesity [72,73]. In addition, insulin resistance and glucose intolerance were observed in mice lacking 5-HT_{2C}Rs [72]. A subsequent study demonstrated that anorexigenic effects of serotonin were mediated in part by the 5-HT_{2C}Rs in arcuate nucleus POMC neurons [74]. Interestingly, reexpression of 5-HT_{2C}Rs only in POMC neurons reversed the hyperphagia/obesity and insulin resistance

observed in 5-HT_{2C}R null mice [75,76]. Similar to the effects of leptin on POMC neurons, serotonin stimulates ARC POMC neurons resulting in the release of α-MSH and subsequent activation of the central melanocortin pathway [64,74,77]. Thus, available data suggest that the effects of serotonin on energy and glucose homeostasis are mediated in part by the 5-HT_{2C}Rs in POMC neurons and the activation of melanocortin signaling.

A recent study described a 5-HT_{2C}R-induced suppression of $GABA_B$-activated G protein-gated inwardly rectifying K^+ (GIRK) channels in guinea pig POMC neurons [78]. These data supported a possible role for GIRKs in the 5-HT_{2C}Rs-induced activation of ARC POMC neurons; however, the cellular mechanisms underlying the activation of POMC neurons by 5-HT_{2C}Rs have been unknown. Sohn et al. [79] recently extended these initial observations and found that 5-HT_{2C}R agonists blunted a $GABA_B$-induced GIRK conductance in murine POMC neurons, which was similar to the results obtained in guinea pig. However, POMC neurons from GIRK1 knockout mice were still depolarized by 5-HT_{2C}Rs [79], and further experiments demonstrated that activation of 5-HT_{2C}Rs stimulates a phospholipase C (PLC)-mediated activation of the TRPC channels which ultimately led to a depolarization of POMC neurons (Fig. 1b). Importantly, prior work has suggested that 5-HT_{2C}Rs specifically in POMC neurons mediate the anorexigenic effects of serotonin [76]; thus, these data may provide a cellular correlate to the acute effects of serotonin to reduce food intake.

Opposite to the effects of serotonin on ARC POMC neurons, ARC NPY/AgRP neurons are inhibited by serotonin via activation of serotonin 1B receptors (5-HT_{1B}Rs) [64]. Although the cellular mechanisms underlying the inhibition of NPY/AgRP neurons by 5-HT_{1B}Rs is still uncertain, the 5-HT_{1B}R-induced inhibition of NPY/AgRP neurons is believed to contribute at least in part to the suppression of inhibitory inputs to adjacent ARC POMC neurons [64]. Heisler et al. also demonstrated that acute administration of 5-HT_{1B}R agonists reduced food intake in a melanocortin-dependent manner. Moreover, recent work suggests that most but not all of the anorexigenic effects of d-FEN are mediated via 5-HT_{2c}Rs in POMC neurons and downstream MC4Rs in the paraventricular nucleus and/or amygdala, thus supporting a partial role for 5-HT_{1B}Rs in the serotonin-induced anorexia [80]. As highlighted by the recent work of the Lowell and Elmquist laboratories, this potential indirect regulation of ARC POMC neurons by serotonin may be an important mechanism to disinhibit/activate POMC neurons and thus might contribute to the anorexigenic effects of serotonin in the central nervous system. Although much progress has been made, future studies will undoubtedly expand our current understanding of how 5-HT_{1B}Rs on ARC NPY/AgRP neurons may ultimately regulate energy and glucose balance.

Central Melanocortin Pathways Differentially Regulate Energy and Glucose Balance

Cone and Colleagues first cloned the melanocortin receptors and defined the melanocortin receptors (MC3R/MC4R) in the regulation of energy and glucose homeostasis [5,17,81]. Although mice deficient for MC3Rs are modestly obese, mice deficient for MC4Rs exhibit profound obesity with marked hyperphagia, hyperglycemia, and hyperinsulinemia even on a chow diet [21]. Importantly, mutations in MC4Rs result in a similar obesity syndrome in humans and is now recognized as the most common monogenic form of human obesity described to date [82–85].

Activity-dependent release of α-MSH from POMC neurons activates MC3Rs and MC4Rs and results in a potent inhibition of food intake [5–8]. Importantly, MC4Rs are expressed by distinct central nuclei in rodents [86,87]. In order to delineate a role of MC4Rs in these CNS nuclei, recent studies have relied on genetic manipulations within several regions involved in the regulation of energy and glucose balance. Two recent studies used mouse models which expressed a loxP-modified, null MC4R allele [88,89]. Expression of Cre recombinase restored the endogenous expression of the MC4R allele selectively where Cre recombinase is expressed. The first study targeted MC4Rs primarily in the paraventricular hypothalamus (PVH) and a subpopulation of neurons in the amygdala [88]. Restoration of MC4R expression specifically in the PVH and the amygdala prevented the majority of the obesity observed in MC4R null mice. Importantly, reexpression of MC4Rs specifically in PVH and amygdala completely rescued the hyperphagia observed in MC4R null mice. Interestingly, these findings agreed with a previous observation that hyperphagia and obesity resulted from the haploinsufficiency of sim1 gene, which is a transcription factor regulating PVH development [90]. However, no apparent changes in energy expenditure were observed in these mice. Together, these findings suggest an important role of sim1 and MC4Rs expressed in the PVH and/or amygdala in regulating food intake and body weight independent of alterations in energy expenditure.

A subsequent study used a mouse model to study the role of MC4Rs within all cholinergic neurons as well as another mouse model to study the role of MC4Rs specifically in cholinergic neurons of the hindbrain which included neurons of the NTS and the dorsal motor nucleus of the vagus nerve [89]. Restoration of the endogenous MC4Rs in all cholinergic neurons normalized body weight and energy expenditure without altering food intake. Moreover, hyperglycemia and hyperinsulinemia was normalized while hepatic insulin action and insulin-mediated suppression of hepatic glucose production showed improvement. Although restoration of MC4Rs in hindbrain cholinergic neurons was sufficient to attenuate

hyperinsulinemia; hyperglycemia was only modestly affected and energy expenditure and body weight remained unchanged. These findings suggest a dissociation of melanocortin signaling as it relates to feeding behavior and energy and glucose homeostasis, and thus supports the concept that distinct melanocortin pathways may differentially contribute to these homeostatic modalities.

A Role for POMC Neuronal Heterogeneity

Interestingly, an increase in serum leptin results in both an increase in POMC gene expression and the neuropeptide α-MSH [91–93]. Although, it is intriguing that selective deletion of LepRs in POMC neurons fails to affect food intake [46], acute pharmacological administration of leptin results in a reduction in food intake and body weight which may be mediated at least in part via PI3K activity within ARC POMC neurons [57]. On the other hand, reexpression of 5-HT_{2C}Rs in POMC neurons reversed the hyperphagia observed in 5-HT_{2C}R null mice [76]. These findings suggest that LepRs and 5-HT_{2C}Rs have distinct roles in POMC neurons and until recently it has been unclear whether these divergent effects are mediated by similar or distinct POMC neurons. A recent paper demonstrated that POMC neurons were activated by either leptin or 5-HT_{2C}Rs agonists, however not a single ARC POMC neuron was activated by both peptides [79]. These data suggest that the acute effects of leptin and 5-HT_{2C}R agonists are anatomically segregated in ARC POMC neurons (Fig. 1b). Importantly, POMC neuronal heterogeneity was recently introduced by the demonstration that the acute effects of leptin and insulin were segregated to distinct subpopulations of ARC POMC neurons [94]. These data were further supported by a separate study which reported that while the segregation of the acute leptin and insulin effects on POMC neurons may not be absolute, a subpopulation of ARC POMC neurons acutely responded to leptin but not insulin [61]. Taken together, these data potentially support three functionally heterogeneous POMC neuronal subpopulations.

What is the physiological significance of heterogeneous POMC neurons? As outlined in the current review, LepRs in ARC POMC neurons are required for the regulation of body weight, energy expenditure, and glucose balance; however, direct leptin action on POMC neurons fails to significantly influence feeding behavior. Opposite to the requirement of LepRs, 5-HT_{2C}Rs expressed by ARC POMC neurons are required for the regulation of body weight, insulin sensitivity, and food intake; however, energy expenditure seems to be unaffected by 5-HT_{2C}Rs in POMC neurons. Additionally, MC4Rs in the PVH and/or amygdala are important in the regulation of feeding behavior which contributes to body weight, while MC4Rs in cholinergic neurons regulate body weight and energy expenditure without altering food intake. Moreover, MC4Rs in cholinergic neurons are involved in the regulation of glucose and insulin levels as well as hepatic insulin action. MC4Rs in cholinergic neurons also regulate hepatic insulin resistance; an effect shared with MC4Rs in hindbrain cholinergic neurons. Together these data suggest that leptin and serotonin may alter the activity of a distinct heterogeneous population of ARC POMC neurons which may ultimately be coupled to diverging melanocortin pathways that regulate energy and glucose homeostasis (Fig. 1b).

Summary

POMC neurons of the arcuate nucleus are direct targets of various hormones and neurotransmitters. The effects of leptin and serotonin in energy and glucose homeostasis are mediated at least in part by the LepRs and 5-HT_{2C}Rs expressed by POMC neurons. Recently, the cellular mechanisms for regulating the activity of POMC neurons by these receptors were identified. Both LepRs and 5-HT_{2C}Rs in POMC neurons are critical regulators of body weight and glucose balance; however, recent work has described a divergent role for these peptides in ARC POMC neurons as it relates to the underlying alterations of energy and glucose metabolism. Moreover, recent studies suggest all POMC neurons are not identical but several heterogeneous POMC neuronal populations exist. The possibility that different subpopulations of POMC neurons may constitute distinct melanocortin pathways is an exciting area for future investigation.

Acknowledgments We gratefully acknowledge Joel K. Elmquist D.V. M, Ph.D. for insightful comments on this review. This work was supported by the American Diabetes Association (7-11-MN-16) to J.-W.S. and the National Institutes of Health (K01 DK087780) to KWW.

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