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Hormonal regulation of the arcuate nucleus melanocortin system

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1. ABSTRACT

Over the past century, the hypothalamus has emerged as one of the critical sites involved in energy homeostasis. Degeneration studies in rats performed some six decades ago, first led to identifying hypothalamic subregions controlling food intake and body weight. The idea that the central nervous system (CNS), and the hypothalamus in particular, are key in metabolism regulation was reinforced by the discovery of leptin in 1994. Since the identification of leptin, enormous progress has been made in the understanding of the regulation of hypothalamic and extrahypothalamic brain regions that control food intake and energy expenditure by peripheral signals such as hormones. An important challenge is to decipher these complicated interactions between peripheral signals and neuronal circuits to better understand the etiology of metabolic disorders and to identify opportunities to intervene with pharmacological treatment. In this review, we focus on the hormonal regulation of the neuronal circuits of the arcuate nucleus of the hypothalamus: the melanocortin system.

2. HYPOTHALAMIC ARCUATE NUCLEUS CONTROL OF ENERGY METABOLISM

The hypothalamus consists of several nuclei involved in the regulation of food intake. One of the most studied hypothalamic nuclei is the arcuate nucleus (ARC). The ARC, located at the medial base of the hypothalamus around the third ventricle, contains at least two distinct groups of neurons controlling energy balance: the orexigenic neuropeptides; agouti-gene-related protein (AgRP) and neuropeptide Y (NPY) containing neurons, and the anorexigenic neuropeptides; pro-opiomelanocortin (POMC) and cocaine and amphetamine-regulated transcript (CART) expressing neurons. Both the AGRP/NPY and the POMC/CART neurons have been identified as main regulators of appetite, satiety, and the regulation of energy expenditure. Because of their proximal location to the median eminence (ME) where the blood brain barrier (BBB) is not present (1), these neurons are responsive to many peripheral signals including leptin, insulin, glucocorticoids, ghrelin, thyroid hormones and many other hormones. These

peripheral hormones influence energy homeostasis either by activating or inhibiting the activity of these two antagonistic neuronal populations. The activation of POMC neurons by leptin, for example, triggers the release of α-MSH from their axon terminals, which in turn activates MC4R, leading to suppressed food intake. Simultaneously, leptin suppresses the activity of arcuate nucleus NPY/AgRP neurons (2), which otherwise would antagonize the effect of α-MSH on MC4Rs through the release of AgRP (3). The ARC neurons, also defined as "the melanocortin system" (for their target, the melanocortin receptor 4) or "first order neurons", project to the so-called "second order neurons" in several hypothalamic areas including the paraventricular nucleus (PVN), ventromedial nucleus (VMH), dorsomedial nucleus (DMH), and lateral hypothalamic area (LHA) (4). From these areas, neurons then project to, among others, the nucleus of the solitary tract (NTS) in the brainstem and the dorsomotor nucleus of the vagus (DMV), where the descending hypothalamic inputs are integrated with peripheral afferent inputs from the liver and gastrointestinal tract.

3. LEPTIN

The discovery of leptin represents a milestone in the research of central metabolic regulation, providing a new approach for the understanding of neuronal communication. Since then, other peripheral signals have been identified as primary metabolic signals, but none of them is as closely associated with the central regulation of metabolism as leptin. Leptin is synthesized mainly by adipose tissue, and its circulating levels are directly related to adipose tissue mass (5). High leptin levels signal the presence of sufficient energy stores to the area of the brain involved in the homeostasis of energy balance, which, in turn, respond by reducing appetite and increasing energy expenditure (6). Several studies support the hypothesis that most, if not all, of the biological effects of leptin are likely to originate in the hypothalamus. In support of this, leptin receptors (OB-R) are highly expressed in this region of the CNS (7).

Leptin is an emerging pleiotropic molecule, and its importance in a variety of physiological conditions is illustrated by the complex syndromes exhibited by leptin-deficient *ob/ob* mice and leptin receptor-deficient *db/db*. These mice are not only obese, they show abnormalities in reproductive function, hormone levels, wound repair, bone structure, and immune function (8).

The structure of leptin and its receptor suggest that they are members of the cytokine family (9). The OB-R is alternately spliced into at least five transcripts from the single *db* gene. The encoded proteins are called the long (OB-Rb), short (OB-Ra, -c, and -d), and soluble forms (OB-Re) (10). The short isoform (OB-Ra) is the most abundant isoform found in tissues (11). It has been reported to have signaling capabilities (12), but its complete role is still unclear, and it remains to be determined whether it might also function as a decoy receptor or have a role as a ligand-passing receptor.

Like other cytokines, leptin circulates both in a free form and bound to its soluble receptor OB-Re (13). The complex leptin-OB-Re has been shown to delay leptin clearance from circulation and potentiate its effect on food intake (14).

OB-Rb is the isoform responsible for signal transduction, and is essential in mediating most of the biological effects of leptin (10), (15). The long isoform is highly expressed in the hypothalamus (16), but is also present in peripheral tissues, including those of the immune system such as the spleen, thymus, lung, and leukocytes (17), (18), (19).

OB-Rb is the only isoform containing an extracellular ligand-binding domain, a single transmembrane domain, and a cytoplasmic signaling domain (11). Unliganded OB-Rb exists as a preformed homodimer. Leptin binding alters the conformation of the OB-Rb dimer, enabling activation of Janus kinase/signal transducers and activators of transcription (STAT) as well as mitogen-activated protein kinase signal transduction pathways (16). In common with cytokine-transducing pathways, leptin induces expression of SOCS (suppressor of cytokine signaling)-3 mRNA in the hypothalamus. SOCS proteins are thought to function as inducible intracellular negative regulators of cytokine signal transduction. Accordingly, transfection data suggest that SOCS-3 is an inhibitor of leptin signaling (20).

Recent studies on neuronal circuits that regulate energy metabolism have revealed that the hypothalamus is not a static component of the CNS. The circuit components of energy balance are integrated into a fluid system involving synaptic plasticity (21). It has been shown that a differential synaptic input organization is present on NPY and POMC neurons in leptin-deficient *ob/ob* mice compared to their wild type controls. In accordance with this, different postsynaptic currents onto NPY and POMC neurons were also observed between the two animal groups. On the perikaryal membrane of POMC neurons of *ob/ob* mice fewer synapses were found compared to wild type animals, and in addition, symmetrical putative inhibitory synapses were found to dominate over the asymmetrical excitatory synapses. In the same way, a higher number of synaptic inputs were observed on the parykarya of NPY neurons of ob/ob mice compared to their wild type controls. Of these inputs, the majority were asymmetrical putative excitatory synapses (21). When leptin was systematically administrated to these *ob/ob* mice, the synaptic density rapidly (several hours before the effect of leptin on food intake) normalized to the levels of wild type mice. In addition to leptin, ghrelin, a gut hormone, was also found to affect synaptic input organization onto NPY and POMC neurons of the arcuate nucleus. Contrary to the effects of leptin, ghrelin delivered to wild-type mice induced a rearrangement of the synaptic inputs onto POMC perykarya supporting the inhibitory tone on these neurons (21).

Because synaptic plasticity was also observed in wild-type animals (22), it is reasonable to propose that this is a continuous phenomenon occurring in feeding circuits. During satiety, when circulating leptin concentrations are high and ghrelin low, the synaptic inputs of NPY/AGRP

neurons are dominated by inhibitory connections (satiety or anorexigenic tone) while excitatory synapses dominate on the perykarya of POMC neurons. During hunger, when leptin concentrations diminish and ghrelin levels increase, these connections are rearranged so that excitatory synapses will dominate over inhibitory inputs on NPY/AgRP neurons (hunger or orexigenic tone) and inhibitory synapses will dominate on the perykarya of POMC neurons.

The molecular basis for the hormonal-mediated synaptic plasticity still remains to be clarified. Of course, synaptic plasticity may not directly trigger alterations in postsynaptic response, and does not alone explain changes in metabolic states. Moreover, this model suggests that, depending on peripheral metabolic need, the probability of either activation or inhibition of the melanocortin system may be readily altered by synaptic rearrangements. Further studies of the mechanism(s) underlying hormone-induced plasticity could provide important insights into the regulation of feeding, and potentially into the regulation of other complex behavior.

4. GHRELIN

At the beginning of the twentieth century the stomach was considered responsible for the regulation of food intake. This discarded hypothesis was then validated when ghrelin, a hormone mainly produced by the stomach, was shown to increase food intake and body weight (23), (24). Ghrelin was first identified for its growth hormone-release activity as a result of binding the orphan growth hormone secretagogues receptor (GHS-R) (25) in the pituitary gland. Mainly produced by the stomach as *preproghrelin*, it undergoes post-transcriptional modification to reach the active form required for most of its biological functions, including its neuroendocrine and orexigenic activity. In particular, the acyl-modification on the serine-3 residue with octanoil acid is essential for binding and activating its classical isoform receptor. The GHS-R is alternatively spliced into two different isoforms, GHS-R1a and GHS-R1b (26), (27), and it is reasonable to assume that more isoforms, not yet identified, may mediate different ghrelin actions. GHS-R1a expression has been reported in the hypothalamus and pituitary gland in accordance with its proven role in GH-release and appetite regulation (28), (29). The significance of GHS-R1b still remains to be clarified.

Although ghrelin-producing cells are most abundant in the fundus of the stomach, lesser concentrations of ghrelin are present in other tissues, like the small intestine, kidney, placenta and lungs (for review see 30). A controversial question is how ghrelin affects brain regions from the periphery. Different pathways have been hypothesized. Through the circulation, ghrelin may be transported across the BBB (31), thus reaching brain areas including the hypothalamic arcuate nucleus regions. Alternatively, once released by the stomach it may signal the nucleus of the solitary tract via the vagus nerve (32). In addition, the presence of ghrelin immunoreactivity in hypothalamic neurons has been reported (33), suggesting a third possible pathway involving a direct action from brain-derived ghrelin. Specifically, ghrelin immunoreactivity was found in a particular hypothalamic region around the third ventricle between the PVN, VMH, DMH, and periformical regions, that did not overlap with any of the known neuronal populations involved in energy homeostasis. Ghrelin axon terminals have also been found to strongly innervate arcuate NPY neurons and paraventricular CRH, and TRH neurons (33).

Extensive studies have clearly demonstrated that the arcuate NPY/AgRP neurons are the main target of ghrelin action in the hypothalamus. These neurons express GHS-R (34), are activated (showed by c-fos immunoreactivity, a marker of neuronal activation) following peripheral ghrelin administration (35) and electrophysiological examination has shown a depolarizing effect of ghrelin on NPY neurons (33). In addition to the regulation of energy metabolism through the hypothalamus, recent evidence has revealed that ghrelin may regulate metabolism by affecting other areas of the brain such as the mesolimbic dopaminergic system of the ventral tegmental area (VTA) (36). This area of the midbrain plays a critical role in the mechanisms associated with reward-seeking behavior, including actions to obtain natural rewards like food. In addition, ghrelin has been shown to affect cognitive functions including learning and memory processing (31). This study has demonstrated that ghrelin binding is present in the hippocampus, an area of the brain involved in learning and memory. Through a combination of anatomical, behavioral and electrophysiological and genetic studies, it has been demonstrated that ghrelin increases the excitability of the hippocampal pyramidal neurons with a concomitant increased performance in learning and memory tasks (31). Finally, in addition to the role of ghrelin in growth hormone release, appetite stimulation, and cognitive function, other functions have been reported. Accordingly, with the wide expression of GHS-R in peripheral tissues (37), ghrelin has been shown to increase gastric function, positively affect the cardiovascular system, assist in bone formation , and help regulate immunity, inflammation and reproduction (for review see 30).

5. INSULIN

In the last twenty years the role of insulin within the CNS has attracted increasing attention. As it became evident that the blood-brain barrier was transporting insulin, it also became clear that insulin played a role in pleiotropic effects both peripherally and centrally. The observation that the insulin receptor (IR) is widely expressed in the brain (38), and that brain selective deletion of the IR results in obesity and hyperphagia (39) has led to the hypothesis that insulin is involved in the central regulation of energy homeostasis. In support of this, when delivered directly into the brain, insulin has been shown to lead to the suppression of food intake and a reduction in body weight (40), (41). In addition, these anorexigenic effects have been shown to be reversed by inhibitory stimuli of insulin signaling (39), (42).

Whereas the peripheral effects of insulin are to decrease the amount of circulating glucose and to increase the energy stored in liver, fat and muscles, in the CNS, together with leptin, insulin decreases the drive to eat

through the melanocortin system (43). Insulin has also been shown to decrease pleasurable responses to food through the limbic system, nucleus accumbens and VTA (44). Leptin and insulin are part of an elegant negative feedback loop, and they have redundant effects in response to energy excesses. A direct inhibitory effect of insulin on the hypothalamic orexigenic neurons (45), (46), and an excitatory effect on the anorexigenic neurons has been shown (43), which is analogous to the leptin regulation of the melanocortin system.

Leptin and insulin receptors colocalize in neurons in the same hypothalamic regions, and, although, they are part of two different receptor families, downstream the two signal pathways converge at the level of phosphatidylinositol-3 kinase (PI3K) (47). It has been shown that central injections of a specific PI3K inhibitor can block the anorexigenic effect of both insulin and leptin (48), (49).

The insulin receptor is a membrane-bound tyrosine receptor. In the CNS, after insulin binding, IR autophosphorylates recruiting IRS proteins and subsequently activating PI3K and mitogen-activated protein kinase (MAPK) cascades. Phosphatidylinositol-3,4,5-triphosphate (PIP3), generated by PI3K, then binds phosphoinositide-dependent protein kinase 1 (PDK1) that consequently phosphorylates AKT protein kinase. Activated AKT then enters the nucleus to regulate neuropeptide expression through the activation or exclusion of FOXO1 (for review see 50).

Recent research has focused attention on alternative pathways in response to IR activation that involve ATP-sensitive potassium channels (K_{ATP}). K_{ATP} channels in the brain may be regulated by insulin through the activation of PI3K (51), and the secretion of neuropeptides involved in the melanocortin system may be regulated by insulin-induced activation of the PI3K pathway. Insulin action on the NPY/AgRP-containg neurons induces hyperpolarization through the opening of K_{ATP} channels (52). Consequently, neuronal inhibition could be responsible for the inhibition of the secretion of orexigenic peptides. Although different mechanisms have been postulated (for review see 53), more analyses are necessary to further elucidate these complex signaling pathways.

Interestingly, the central effects of insulin also include the regulation of glucose metabolism in the periphery. Central injection of insulin reduces glucose production in the liver, whereas, blocking insulin signaling with an inhibitor of central K_{ATP} channels, diminishes the ability of insulin to suppress peripheral glucose output (54). This regulation may involve electric transmission via the autonomic nervous system, since transection of the vagal nerve abolishes this effect (55).

The hypothalamus orchestrates and coordinates the neuroendocrine regulation of energy balance through a complex pathway via afferent signals received from the periphery depending on nutrient status, and efferent pathways including the sympathetic nervous system, which promotes energy expenditure, and the parasympathetic nervous system, which promotes energy storage (for review see 56). The involvement of insulin in both of these afferent and efferent pathways may help provide some insight into how insulin disregulation may mediate the development of obesity-related insulin resistance.

6. THYROID HORMONES

Thyroid hormones have been shown to play major roles during development as well as in adulthood. An important contribution of the thyroid hormones is to assure appropriate metabolism at both the cellular and whole organism levels. Various transcriptional and non-transcriptional effects of thyroid hormone have been identified that may alter cell and tissue metabolism (57), (58), (59).

Thyroxine (T4) is the major form of thyroid hormone secreted by the thyroid gland. Once produced, T4 travels in the bloodstream linked to the carrier proteins, T_4-binding globulin (TBG), transthyretin, and T_4-binding prealbumin. The conversion of T4 into the active form triiodothyronine (T3) is catalyzed by iodothyronine deiodinases and is required for peripheral and central function. In target tissues, deiodination represents the most important pathway for the activation as well as the deactivation of thyroid hormone and the regulation of these deiodinating enzymes occurs in a tissue-specific manner (60).

The entry of thyroid hormones into target cells requires membrane transporters (61). Different thyroid hormone transporters have been identified, with a wide range for different ligands. Only OATP1C1 and MCT8 are thyroid-hormone-specific and, at least in humans, have a high preference for T4 and T3, respectively. In the CNS, T4, is transported by OTP1C1mainly through the blood-brain barrier (62), and to a lesser degree through the blood-CSF barrier using both OTP1C1 and MCT8 transporters (63). Once in the tissue, T4 is translocated into astrocytes and tanycytes and subsequently converted into its active form, T3, by type 2 5'iodothyronine deiodinase (DII) (64), (65), (66).

T3 reaches its nuclear target site of action within the neurons, via the MCT8 membrane transporter (67). MCT8 was first identified (68) on chromosome X in a region associated with X-linked diseases. Its biological function as a specific thyroid hormone transporter was only recently discovered (69). A mutation or deletion in the MCT8 gene, despite strongly elevated T3 levels in circulation, results in severe tissue-specific hypothyroidism. In particular, patients with an inactivation of MCT8 were reported to have a severe neurological phenotype (70), (71), suggesting that MCT8 plays an essential role in the CNS as a route for T3 to enter neurons. The MCT family is composed of 14 members based on sequence homologies. The first four members have been characterized as transporters of monocarboxilates. MCT8 and MCT10 show 49% amino acid homology and transport aromatic amino acid derivatives. All of the other members are still orphan transporters and their ligands

remain to be determined. In the CNS, MCT8 is almost exclusively expressed in neurons of the neocortex, hippocampus, basal ganglia, amygdala, Purkinje cells of the cerebellum, and hypothalamus. In the latter, MCT8 was also found in tanycytes lining the third ventricle (67). The regulation of MCT8 remains to be identified. Recent evidence reports that there are modifications of MCT transporter family expression in pathological situations (72). Information about MCT8 regulation will allow better insight into the action of thyroid hormone.

T3 activity could be regulated in specific tissues by its access into neurons. Once transported into neurons, T3 actions are mediated by nuclear receptors (73). Thyroid Hormone Receptors (TRs) are encoded by two separate genes, TRα and TRβ, located in different chromosomes (17 and 3, respectively, in humans). Alternative splicing from each gene generates multiple TR isoforms, including TR α1, TR α2, and TR α3 from the TRα gene, and TRβ1 and TRβ2 from the TRβ gene (74). TR forms a heterodimer with the retinoid X receptor (RXR) and binds to T3 responsive elements (TREs) in the promoter region of thyroid hormone-responsive genes. Binding of T3 to its receptor induces the release of corepressors and the recruitment of coactivator, resulting in the stimulation of transcription.

The hypothalamo-pituitary-thyroid (HPT) axis is essential for normal development, differentiation, and metabolic balance. Under euthyroid conditions, thyrotropin-releasing hormone (TRH), a neuropeptide produced in the paraventricular nucleus of the hypothalamus, stimulates the pituitary gland to secrete thyroid-stimulating hormone (TSH). TSH, in turn, activates the thyroid gland to release thyroid hormones which, in turn, will feedback to the hypothalamus. Interestingly, within the hypothalamus the major source of DII, the thyroid hormone activating enzyme, is expressed in the arcuate nucleus-median eminence region (75); (64), (65), (76), (77). The cell types that produce DII are glial cells and astrocytes, which are homogeneously distributed within the arcuate nucleus, and tanycytes, which are predominately located on the floor and ventrolateral walls of the third ventricle. Thus, tanycytes may provide a bidirectional movement of substances between the CSF in the third ventricle and the blood in the vascular elements of the arcuate nucleus and/or median eminence. These glial cells provide an extensive network of cellular processes in the ARC which may suggest a paracrine action on PVN-projective ARC neurons via the production of thyroid hormones.

In addition to expressing high levels of DII activity, the arcuate nucleus has also been found to contain an abundant population of thyroid receptor-producing neuronal nuclei (78), as well as populations producing various regulatory peptides and neurotransmitters such as neuropeptide Y, opioid peptides, growth hormone releasing hormone and dopamine, all of which are known to influence the production and release of TRH (79), (80), (81). The existence of a monosynaptic pathway between the arcuate nucleus that contains DII-producing glial cells, and the paraventricular TRH neurons that project to the median eminence with direct access to fenestrated capillaries has been demonstrated (76). In has also been shown (65), (82) that the arcuate nucleus NPY/AgRP neurons provide a massive inhibitory input on TRH cell bodies and proximal dendrites via symmetric and thus putative inhibitory synapses. In addition, it has also been reported that TRH cells are symmetrically contacted by nerve terminals containing AgRP, which is co-produced in the NPY arcuate neurons (83).

Hypothyroidism due to failure of the thyroid gland induces a rise in hypothalamic TRH levels, which, in turn, triggers release of TSH from the anterior pituitary. This classic negative feedback of the thyroid axis is paradoxically reversed during fasting. Food deprivation is known to decrease thyroid function, and surprisingly low levels of circulating T4 and T3 coincide with the suppression of hypothalamic TRH production and release in the hypothalamic paraventricular nucleus and median eminence (84). A differential expression of DII during fasting seems to be responsible for the activation of the inhibitory NPY/AgRP neurons and an inhibition of the excitatory α-MSH projections onto paraventricular TRH neurons, thereby explaining the paradox that suppressed levels of circulating T4 and T3 parallel the inactivation of TRH. Specifically, its has been demonstrated that during fasting DII mRNA and activity are elevated in the arcuate nucleus-median eminence region (66). This elevation in DII production and activity is regulated by an inverse shift in circulating levels of leptin and corticosterone (85). The activation of DII, thus, induces an overproduction of T3 in the hypothalamus (86). This "local hyperthyroidism" could then affect and alter neuropeptide expression in leptin-responsive arcuate neurons that project to paraventricular TRH neurons. This, in turn, could cause the down-regulation of TRH and a reduction in the release of TRH from the axon terminals around the portal vessels in the median eminence.

The signaling modality by which T3 affects arcuate neurons in the regulation of the thyroid axis has recently been delineated (87). It has been largely demonstrated that in peripheral tissues, uncoupling protein expression and activity are regulated by T3 (88). Uncoupling proteins (UCPs) are a subfamily of mitochondrial anion carriers first discovered in brown adipose tissue, where they play a well-described role in thermogenesis. UCPs are located in the inner membrane of the mitochondria, and their primary function is thought to be to leak hydrogen protons from the intermembrane space to the matrix of the mitochondria (89), (90). In the individual mitochondrion, these proteins, through this process, may deprive the driving force of ATP synthase from catalyzing ATP synthesis, dissipate energy in the form of heat, diminish the production of superoxides, and decrease the entry of calcium into the mitochondrial matrix (91).

Five members of the UCP family that differ in tissue distribution, regulation and physiological roles have been discovered. UCP1 (92) and UCP3 (93) are expressed only in peripheral tissues, and UCP4 and BMCP1 are predominantly expressed in the central nervous system (94), (95), while UCP2 is expressed in muscle, adipose tissue, spleen, and the central nervous system (96), (97), (98), (99).

In the brain, UCP2 is expressed predominantly in neuronal populations of the brain stem and hypothalamus involved in the central regulation of autonomic, endocrine, and metabolic processes in both rodents and primates (97), (98), (99). The presence of decreased mitochondrial energy coupling efficiency (increased proton leak) in UCP2-containing brain regions supports the hypothesis that a thermogenic mechanism is intrinsic to distinct neuronal pathways (98). In correlation, brain tissue temperature in UCP2-containing brain regions has a significantly higher local temperature when compared to other sites or to the core body temperature. Other brain regions lacking in UCP2, such as the striatum and thalamus, exhibit a significant mitochondrial proton leak, which is consistent with the subsequent discovery of other putative brain uncouplers, UCP4 and BMCP1 (94), (95).

UCP2 expression has been found in mitochondria of neurons, particularly in axon terminals, suggesting a direct role for interneuronal communication as a trigger for the mitochondrial uncoupling/thermogenic mechanism in circuits involved in the central regulation of homeostasis. Acute heat production in axon terminals could immediately accelerate synaptic transmission by affecting synaptic vesicle formation and traffic, neurotransmitter release and reuptake, and the tertiary structure of neuromodulators as well as directly influencing the postsynaptic membrane potential (98). The involvement of UCP2 in central metabolic pathways is also supported by the finding of the abundant co-expression of UCP2 and NPY in the arcuate nucleus neurons. Moreover, UCP2-containing axons were found to innervate other hypothalamic peptidergic systems, including MCH- and orexin-producing cells that participate in metabolic regulation (98).

In the orexigenic neurons of the hypothalamic arcuate nucleus region an intriguing interplay has emerged between thyroid hormone, T3, and UCP2 in the regulation of the melanocortin system (87). A close proximity between DII-containing, and hence T3-producing, glial elements and components of the melanocortin system, in particular, NPY/AgRP neurons expressing UCP2 has been demonstrated (87). Thus, it is reasonable to assume that arcuate nucleus neurons may be exposed to locally formed T3, and that thyroid hormone may have direct access to the melanocortin system. This provides a basis for neuronal-glial interaction in metabolism regulation as DII activity is upregulated during fasting. Once produced, T3 triggers UCP2 mRNA expression and mitochondrial uncoupling activity in the hypothalamus. The activation of UCP2, in turn, leads to an increase in mitochondrial number (100), (87), that, despite a decrease in ATP production by individual mitochondria, could increase the overall production of ATP.

However, it is not clear whether the increased number of mitochondria is due to a recruitment of these organelles into the cell body or their proliferation. Nevertheless, this mechanism could enhance neuronal activity and thus enable NPY/AgRP neurons to have increased firing after refeeding.

Under fed conditions, leptin, the peripheral anorexigenic hormone, controls the melanocortin system (101). During fasting, orexigenic hormones, such as ghrelin, will reverse the tone of the melanocortin system in which NPY/AgRP neuronal activity will dominate (102). At the same time, elevated corticosteroid levels will induce DII activity (66), (85) and thus, local T3 production (86), which will trigger UCP2 production and activity in the mitochondria of NPY/AgRP neurons. On the basis of these events, it is suggested that by the time of refeeding, activated UCP2 will induce mitochondria proliferation in NPY/AgRP neurons which, in turn, will be a critical factor in maintaining increased firing of these orexigenic cells so that food intake will remain elevated subsequent to refeeding, at a time when neither orexigenic nor anorexigenic circulating signals dominate. This learning/memory process within the melanocortin system entails mitochondrial proliferation and increased available ATP levels in NPY/AgRP neurons. This in turn, enables elevated NPY/AgRP and suppressed POMC neuronal activity despite increasing metabolic signals.

7. GLUCOCORTICOIDS

Glucocorticoids are known to affect all hypothalamic functions including neuroendocrine, autonomic and homeostatic functions. A close relationship between glucocorticoid secretion and feeding, metabolism and energy storage is also well documented (for review see 103). Adrenalectomy (ADX) reduces food intake, fat storage and weight gain (104), (105). In rodents, ADX decreased NPY mRNA expression (106) while corticosterone replacement increased NPY levels (106). The decrease of NPY after ADX occurs despite the fall in plasma leptin and insulin concentrations which in other situations would increase these neuropeptides (107). On the other hand, different genetic models of obesity are associated with an increase in corticosterone levels (for review see 4). During fasting, leptin levels decrease while circulating corticosteroid and ghrelin levels increase. These changes are associated with increased NPY/AgRP and decreased α-MSH neuronal stimulation (4), (102). Systemic administration of leptin to fasted animals has been shown to induce a reduction in corticosterone levels (85), and also restored paraventricular TRH mRNA to the level of intact animals (108). The existence of a glucocorticoid-responsive element in the promoter region of the pro-TRH gene is known (109), and the mechanism of action of glucocorticoids on TRH gene expression has been shown to occur directly through the presence of glucocorticoid receptors on TRH-producing cells (110). However, an indirect effect of corticosterone on TRH levels has been recently demonstrated, adding an additional layer of complexity to the hormonal regulation of hypothalamic neuronal circuits (86), (87).

Corticosterone has been shown to be a critical trigger for the increase in DII activity seen during negative energy balance. The increased DII activity, in turn, was shown to boost hypothalamic T3 production, which is responsible for the upregulation of uncoupling protein 2 activity in NPY/AgRP neurons. The increased UCP2 activity could then induce an increase in mitochondrial density that could be responsible for the sustained activity of these orexigenic neurons during negative energy balance.

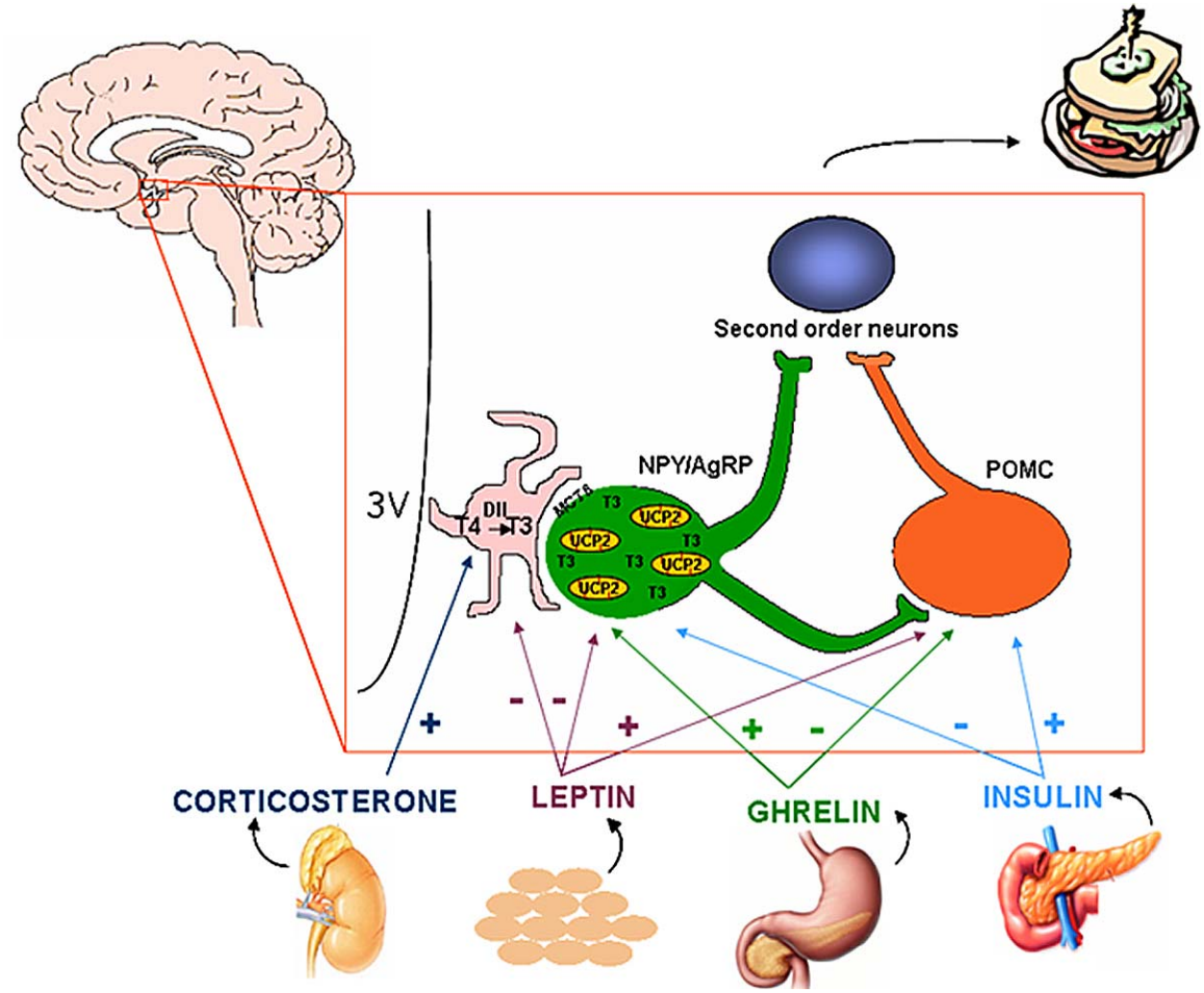

Figure 1. Schematic drawing illustrating the effects of peripheral hormones such as glucocorticoids, leptin, ghrelin and insulin on the hypothalamic melanocortin system. Circulating leptin and insulin activate POMC neurons, and simultaneously suppress the activity of NPY/AgRP neurons, leading to a reduction in food intake. During negative energy balance such as fasting, ghrelin, produced by the stomach, reverse the tone of the melanocortin system in which NPY/AgRP neuronal activity will dominate. At the same time, elevated corticosteroid levels and decreased leptin levels trigger DII activity in glial cells inducing an increase of local T3 production. This local hyperthyroidism leads to UCP2 production and activity in the mitochondria of NPY/AgRP neurons which will sustain an elevated neuronal activity of these orexigenic neurons.

The activation of NPY/AgRP neurons, which strongly project to paraventricular TRH neurons (65), (76), could then decrease the levels of TRH mRNA. In support of this mechanism, we found that adrenalectomy prevented the increase in DII activity reported in fasted animals, while corticosterone replacement restored the enzymatic activity in a dose-dependent manner (85).

The regulation of hypothalamic DII by glucocorticoids needs to be further investigated. The exact manner by which glucocorticoids act on glial cells producing DII requires additional study. There is evidence that glucocorticoids directly target glial cells. Indeed, glucocorticoid receptors have been found to be localized in glial cells as well as in neurons with an intense immunoreactivity in the hypothalamus (111).

8. PERSPECTIVE

The aforementioned examples of hormonal regulation of the hypothalamic melanocortin system (Figure 1) do not alone account for all of the peripheral signals that regulate metabolism via the arcuate nucleus. Many other signals, including other hormones such as estrogen (112), and nutrients such as glucose (113), free fatty acids (114) and amino acids (115) have been shown to regulate food intake and energy expenditure by acting on the NPY/AgRP and POMC neurons of the hypothalamus. It is also important to consider the possible interactions between the intracellular signaling pathways activated by these hormones and nutrients, and how these pathways may affect neuronal electrical activity, since this will ultimately be responsible for the release of neuropeptides and neurotransmitters.

Furthermore, intercellular communication represents another important aspect of the regulation of metabolism that needs to be taken into account. In order to correctly assess neuronal activity, synaptic organization (the inputs and outputs of neuronal populations) must be considered and further elucidated. Overall, an interdisciplinary approach with the help of genetically manipulated animal models is needed in order to address these issues which may ultimately lead to the development of novel therapeutic approaches for the treatment of obesity and type-2 diabetes.

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