Neuroendocrinology of Energy Balance

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Abstract

In the past decades, the spiraling obesity epidemic has renewed the interest of basic scientists in the control of hunger and satiety, food intake and energy expenditure, and body weight regulation by the central nervous system. The discovery of the adipose-derived satiety hormone, leptin, in 1994 greatly advanced the neuroscience of obesity by enabling detection and characterization of the – largely hypothalamic – neurocircuits that underpin feeding behavior and energy balance regulation. A number of circulating factors that affect the energy

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balance at the central level have subsequently been discovered in the adipose organ, the gastrointestinal tract, and the endocrine pancreas or their mechanisms of action have been characterized. Although several major pieces of the picture are still missing, the available data suggest that energy balance homeostasis is achieved at the central level by hypothalamic and brainstem neurocircuits which integrate metabolic stimuli with cognitive, hedonic, and emotional cues, regulating energy use and storage and body weight homeostasis through behavioral, autonomic, and endocrine responses. These extremely complex and closely integrated neurocircuits are mainly peptidergic and give rise to a highly redundant system. They operate continuously in response to stimulatory or inhibitory hormonal and metabolic inputs coming from the periphery of the body through the circulation. Such crosstalk between "center" and "periphery" is currently a major area of energy balance research. Its elucidation is expected to provide in the near future novel druggable targets for the effective treatment of obesity and related diseases in humans.

Keywords
Hypothalamus · Arcuate nucleus · Solitary tract nucleus · Circumventricular organs · Leptin · Insulin · Ghrelin · Cholecystokinin · Peptide YY · Glucagon-like peptide-1 · Amylin

Introduction

In the whole animal kingdom, the search for nutrients is key to survival. During evolution, animals have developed complex biological systems to search for food, maintain a homeostatic internal metabolic environment, and store energy, mainly in the form of fat, to overcome periods of fasting and to sustain energy-intensive behaviors such as reproduction. Conceivably, a greater ability to store energy and withstand prolonged fasting has also played a role in the differentiation of mammalian brain and behavior, partly releasing individuals from the constant quest for food to pursue increasingly complex tasks, including exploration of the physical environment and social interaction.

More than 50 years ago, the "thrifty genotype" hypothesis suggested that genes favoring energy storage and reduced energy expenditure were positively selected during the evolutionary history of mammals, including humans, and enhanced survival in an energy-poor environment (Neel 1962). Thus, despite marked differences among species and individuals, the mammalian genotype is evolutionarily geared to a highly efficient use of food-derived energy (Sellayah et al. 2014). However, in the modern obesogenic environment these genetic advantages have become a problem, since the virtually limitless availability of calorie-rich food and the diffusion of sedentary lifestyles have led to a severe epidemic of obesity, type II diabetes, and metabolic disease. From a therapeutic viewpoint, overriding this highly efficient evolutionarily selected system has proved extremely difficult.

The central nervous system (CNS) plays a crucial role in all energy balance-related processes, from the hunger sensation and search for food up to energy expenditure and/or accumulation. This review begins with a historical section describing the identification of the main brain areas and molecules involved in mammalian energy balance regulation; it then examines the brain areas most closely involved in energy balance control; and finally presents a systematic overview of the most important peripherally produced hormones which, by acting at distinct brain sites, regulate different aspects of the energy balance. Understanding the mechanisms underpinning energy balance regulation has helped devise some obesity treatments and is likely to prove even more useful in the future. Yet, there are at present few and only mildly effective pharmacological treatments for human obesity, and the sole therapeutic option available to the morbidly obese is bariatric surgery. In the past few years, a greater understanding of body weight homeostasis and appetite regulation has provided an impressive list of potential druggable targets. This knowledge and the intense research effort currently under way are likely to lead to the development of successful single or combination treatments for obesity.

The Birth of the Neuroendocrinology of the Energy Balance: A Historical Perspective

In the mid-nineteenth century, pituitary tumors were known to lead to obesity and hypogonadism; this clinical condition was called Frohlich's syndrome. At the time, the excessive fat accumulation in such patients was attributed to endocrine abnormalities due to pituitary gland dysfunction. The general belief that obesity was primarily related to pituitary dysfunction was the prevailing view until the first decades of the twentieth century, when it was first found that obesity often developed in patients with tumors at the base of the brain, near but not extending to the pituitary. In the same years, further challenges came from experimental studies showing that in dogs and rats, lesion of the basomedial hypothalamus often resulted in obesity, whereas hypophysectomy without additional hypothalamic damage did not (Elmquist et al. 1999; King 2006).

It is generally accepted that the modern era of brain research in feeding behavior began in 1939, with the adaptation of the Horsley-Clarke stereotaxic instrument for use in rat studies. This apparatus allowed reaching specific sites of the brain of anesthetized animals to introduce fluids or implant cannulae. Thus, bilateral electrolytic lesions of the rat hypothalamus, sparing the adjacent pituitary gland, demonstrated that bilateral damage to the ventromedial portion of the tuberal hypothalamus induced hyperphagia and obesity (Hetherington and Ranson 1940). A few years later, bilateral lesions in the adjacent lateral hypothalamus were shown to result in severe anorexia, weight loss, and even death by starvation (Anand and Brobeck 1951). These studies firmly established a crucial role of the hypothalamus in body weight regulation, and the hypothesis that obesity was the result of a pituitary dysfunction was shelved. Moreover, these investigations suggested the presence in the hypothalamus of two centers regulating food intake and energy balance and

exerting opposite actions: the satiety center, corresponding anatomically to the ventromedial hypothalamus, and the feeding center, corresponding to the dorsal and lateral hypothalamus. These seminal experiments sketched a massive, complex, bilateral, and redundant role of the brain in energy balance regulation. In 1954, the discovery of the hypothalamic satiety and feeding centers suggested to Eliot Stellar his dual-center hypothesis for motivated behavior, where the vast majority of motivated behaviors, including hunger, thirst, reproduction, and aggressiveness, would be produced by the reciprocal and opposing action of excitatory and inhibitory brain centers (Stellar 1954).

In the mid-twentieth century, Gordon Kennedy was the first to suggest that circulating signals generated by peripheral organs in proportion to their energy stores could influence food intake and energy expenditure in a coordinated manner to regulate body weight (Kennedy 1950). The hypothesis obtained some experimental evidence from parabiosis studies, complex experiments where sharing of the blood supply between two animals was achieved through a surgical connection. In particular, lesion of the ventromedial hypothalamus (the "satiety center") of one animal of the pair resulted in its gaining weight, while the other animal refused food and eventually died; however, lesion also of the second animal's ventromedial hypothalamus resulted in its overeating and ultimately in obesity (Hervey 1959). Collectively, these experiments were the first to suggest that as yet unidentified blood-borne satiety factors produced by the obese animal affected food intake and that an intact hypothalamus was required for their action.

The question remained as to which signal(s) the hypothalamic centers could sense in the blood in order to regulate food intake and body weight. Jean Mayer advanced a highly popular theory involving glucose as the signal (Mayer 1955). According to this hypothesis, glucose metabolism in certain hypothalamic cells generates a signal to the brain areas controlling appetite and food intake. When, after prolonged fasting or physical exercise, glucose levels decrease, the impaired glucose metabolism in these cells induces the hunger sensation and the animal begins to search for food, and eats if food is available. As eating progresses, blood glucose progressively augments and is again metabolized by the same hypothalamic neurons, which elicit the satiety sensation and halt the eating. At the experimental level, important support for Mayer's glucostatic theory was provided by the voracious eating of animals administered 2-deoxyglucose, a toxic molecule that enters cells along with glucose but cannot be oxidized to produce ATP, thus impairing cellular energy metabolism (Smith and Epstain 1969). We now know that mammals feed well before blood glucose declines, and it is generally believed that glucose levels have little to see with the physiological regulation of feeding. However, it should be noted that glucoprivic eating is an acute emergency response to a severe energy deficit of the body, and that it also occurs in pathological conditions such as severe diabetes, where intracellular glucose reduction prompts an urgent search for sugar-rich food.

In the 1960s, stereotaxic studies involving the ablation of hypothalamic connections or injection of neurotransmitters into hypothalamic sites showed that extrahypothalamic areas also play an important role in the central regulation of the energy

balance and demonstrated the role of the neurotransmitters acetylcholine and noradrenaline in the neural regulation of feeding.

A pivotal discovery in 1973 showed that the duodenal peptide cholecystokinin (CCK) acted as a meal-generated circulating satiety factor (Gibbs et al. 1973). CCK was thus the first gut hormone found to have an effect on appetite. In subsequent years, the characterization of its mechanism of action highlighted the role in feeding regulation of brainstem centers, which are now defined as the dorsal vagal complex (DVC) of the brainstem.

In the late 1960s, Coleman had identified a naturally genetically obese (*ob*/*ob*) and a naturally genetically diabetic (*db*/*db*) mouse strain. Their phenotype was characterized by massive overeating, obesity, insulin resistance, and impaired sexual maturation leading to infertility. Parabiosis experiments establishing cross-circulation between the two strains allowed Coleman and his colleagues to infer that the *ob*/*ob* mouse lacked a circulating compound capable of preventing obesity, while the *db*/*db* mouse lacked the receptor for such factor (Coleman 1978). In the early 1990s, the advent of molecular genetic techniques allowed identifying the gene of Coleman's factor, whose product induced a strong satiety effect by acting on the CNS: the factor was named leptin (Zhang et al. 1994). This discovery spurred intense experimental work on the neural mechanisms of energy balance regulation, leading to the identification and characterization of mammalian neuronal circuits and neurotransmitter systems regulating energy intake and expenditure at the hypothalamic and extra-hypothalamic level and ensuring energy balance and body weight homeostasis. Importantly, the discovery of leptin also changed the scientists' view of the adipose organ, from a mere energy depot to an active endocrine organ (see ► Chap. 3, "The Adipose Organ").

The "Central Anatomy" of Feeding Behavior and Energy Balance Control

Feeding is a highly complex behavior that massively involves the brain by recruiting sensory, motor, attentive, cognitive, emotional, and reward neuronal systems. This also applies to humans, where recent advances in neuroimaging techniques, such as functional magnetic resonance, allow recording the activity of distinct brain areas during different phases of feeding. Exposure of fasted healthy humans to food-related cues involves activation of brain regions commonly associated to reward and motivation (striatum, pallidum, and midbrain), of areas held to encode visual processing and attention (visual cortex and anterior cingulate cortex), and of areas involved in gustatory (insula, frontal operculum) and oral somatosensory (postcentral gyrus) processing (Burger and Berner 2014). Notably, the reduced activation of reward areas seen in obese subjects exposed to food-related stimuli has generated the notion of hedonic obesity, where defective reward-related responses to food intake may in some patients override the body's energy balance regulation, resulting in overeating, excess fat deposition, and obesity (Lee and Dixon 2017).

In the orchestrated action of the several interconnected brain areas that are involved in such diverse feeding-related functions, the hypothalamus plays the

"conductor," directing, integrating, and blending visceral, endocrine, and behavioral inputs to respond to contingent situations and ensuring the homeostatic control of metabolism.

The hypothalamus, one of the smallest and most ancient parts of the mammalian brain, is found in all vertebrates. It contains highly conserved neural circuits that control a number of basic life functions and behaviors, including the energy balance, fluid and electrolyte balance, thermoregulation, sleep-wake cycles, stress responses, and reproduction. From an anatomical point of view, it is most easily described from the ventral surface of the brain, where it is bounded anteriorly by the optic chiasm, laterally by the optic tracts, and posteriorly by the mammillary bodies. It is divided in two identical halves by the third ventricle, which is located along the midline. Functionally, the hypothalamus is usually divided from rostral to caudal into three portions: (i) the preoptic area, which mainly contains the integrative circuitries for thermoregulation, fever, electrolyte and fluid balance, the wake-sleep cycle, and reproductive behaviors; (ii) the tuberal hypothalamus, with a stalk connecting it to the pituitary gland, which mainly contains the neural circuits for energy balance regulation and endocrine and vegetative responses; and (iii) the mammillary portion, which is believed to be involved in wakefulness and stress responses. Most functions involve a single side of the hypothalamus, whereas some homeostatic functions, such as feeding behavior and energy balance control, recruit the neural circuits bilaterally. The tuberal hypothalamus is thus the most important portion of the hypothalamus for feeding behavior and energy balance homeostasis. It is divided by the fornix into a medial and a lateral part. The medial part contains well-demarcated neuron groups, such as the arcuate nucleus (ARC), the paraventricular nucleus (PVN), the ventromedial nucleus (VMH), and the dorsomedial nucleus (DMH), whereas neurons in the lateral part are more dispersed, do not form distinctive nuclear groups, and are collectively referred to as perifornical and lateral (LH) areas of the hypothalamus.

Besides the tuberal hypothalamus, some brainstem centers in the hindbrain are also crucially involved in feeding behavior and energy balance regulation. They are found on the border between the medulla and the pons and collectively form the DVC. The DVC comprises: (i) the nucleus of the solitary tract (NST), the main sensory relay for the viscera, including the gastrointestinal tract; (ii) the dorsal motor nucleus of the vagus (DMX), which is the source of vagal efferents controlling such visceral responses as gut motility and secretion; and (iii) the area postrema, a circumventricular organ.

The NTS receives afferent fibers from the facial, glossopharyngeal, and vagus nerves which convey gustatory, mechanical, hormonal, and metabolic visceral information. It is reciprocally connected to other brainstem centers, including the area postrema, the DMX and the lateral parabrachial nuclei, and with the hypothalamus, especially ARC and PVN neurons. Thus, the hypothalamic and brainstem feeding centers are anatomically and functionally interconnected.

The circumventricular organs are called "the windows of the brain." They are distinctive areas located in periventricular position, where the absence of the blood-brain barrier (BBB) involves that circulating factors such as hormones and metabolites quickly cross the fenestrated wall of their capillaries and diffuse some way into the extracellular space, affecting the activity of neurons located in circumventricular

organs or in the adjacent brain parenchyma. Interestingly, both the tuberal hypothalamus and the DVC contain a circumventricular organ, respectively the median eminence and the area postrema. Recent experimental evidence has highlighted the role of these two circumventricular organs in regulating the delivery of circulating hormones and metabolites to the ARC and the NTS, respectively.

The nuclei of the tuberal hypothalamus and the DVC are nodal centers in feeding behavior and energy balance regulation and have strong integrative functions. They receive body energy status information from circulating metabolites and hormones – through the circumventricular organs and/or specific carriers in the BBB – and a wide range of sensory inputs such as taste and gastrointestinal information through the vagal afferents to the NTS. Comparison of these inputs to basic references prompts activation of adequate autonomic, endocrine, and behavioral responses to ensure metabolic homeostasis and to meet the energy requirements of the body.

The strong involvement of the tuberal hypothalamus and the DVC in energy balance regulation is clearly demonstrated by c-Fos immunostaining in fasted animals. Feeding is essential for survival, and prolonged fasting involves strong activation of brain activity. c-Fos is the product of an immediate early gene, whose expression in the brain is elicited by a wide range of stimuli (Sheng and Greenberg 1990). Its detection by immunohistochemistry has been extremely useful in identifying the CNS pathways that are activated by several peripheral stimuli. Whereas fed mice display very low, almost undetectable levels of brain c-Fos in neuronal cell nuclei, fasted animals exhibit strong c-Fos nuclear staining in numerous neurons of both the tuberal hypothalamus (Fig. 1) and the DVC (Fig. 2).

Hormonal Signals Involved in the Control of Energy Homeostasis

In the past few years, a large number of circulating hormones, metabolites, and peptides with a role in the energy balance have been characterized. These factors exert a short- and/or long-term regulatory activity on feeding behavior through a concerted action on several distinct areas of the tuberal hypothalamus and/or the DVC. They also affect other brain areas and are involved in other brain functions, a fact that often hampers the characterization of their true role in energy balance regulation. They come from at least three sites: the adipose organ, the gastrointestinal tract, and the endocrine pancreas. Here, the discussion is confined to those factors that in the past few years have been seen to play substantial roles in energy balance homeostasis and whose mechanism of action have proved paradigmatic to understand how the brain regulates feeding behavior, energy consumption, and body weight.

Leptin

Leptin is a peptide hormone produced and secreted by white adipose cells in proportion to the body's fat energy stores (Zhang et al. 1994). Although under certain conditions, it is also produced by other organs and tissues, including

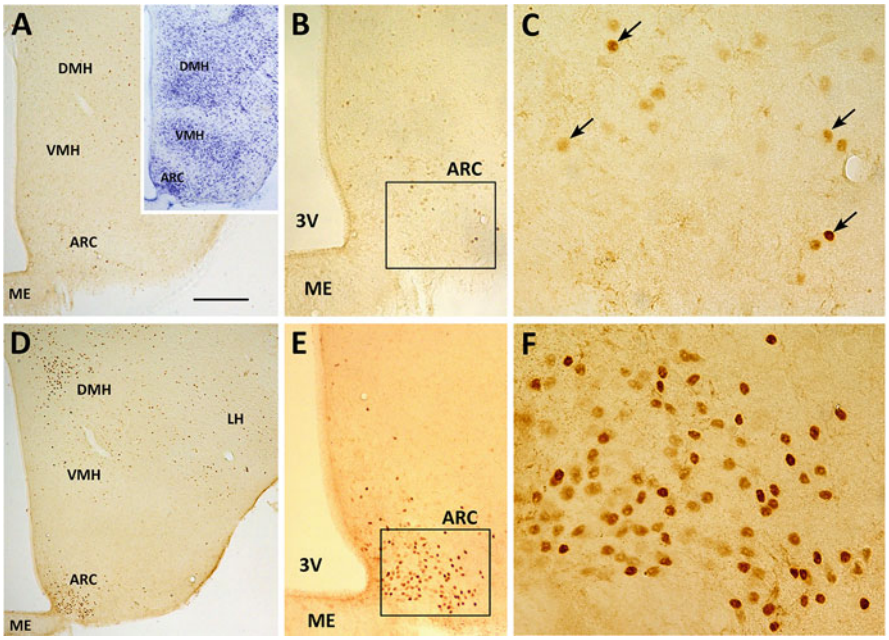

Fig. 1 c-Fos immunohistochemical expression in the tuberal hypothalamus during fasting. In a normally-fed mouse (**a**–**c**), only few and spared neurons located in some hypothalamic nuclei exhibit c-Fos immunoreactivity in their cell nucleus (arrows in **c**). In a mouse fasted for 24 h (**d**–**f**), c-Fos immunoreactive neurons are very numerous and mainly located in medial structures of the tuberal hypothalamus, such as the medial part of the ARC and the DMH, collectively forming what was known as "the feeding center" of the hypothalamus. **c** and **f** are enlargements of the corresponding areas framed in **b** and **e**, respectively. 3V, third ventricle; ME, median eminence. Bar: **a** and **d** 350 μm; inset of **a** 600 μm; **b** and **e** 150 μm; **c** and **f** 30 μm

placenta, mammary gland, stomach, and skeletal muscle, its blood levels closely depend on the secretory activity of white adipocytes and are proportional to their lipid content (Considine et al. 1996). Leptin is a potent satiety factor whose genetic deficiency leads to massive obesity, as seen in *ob*/*ob* mice. Indeed, administration of mouse recombinant leptin to *ob*/*ob* mice reduces food intake and body weight and redresses all the endocrine abnormalities observed in these mice, including hypogonadism, insulin resistance, hypercorticosteronemia, and low levels of thyroid hormones (Ahima et al. 1996). The discovery of leptin was rapidly followed by cloning of its receptor (LepR) (Tartaglia et al. 1995), of which six alternatively spliced isoforms have been identified in mammals. The long isoform (LepRb) contains a fully signaling-competent intracellular domain and is required for most of the central and peripheral effects of leptin. Importantly, the diabetic obese syndrome affecting *db*/*db* mice is due to a mutation of the *LepRb* gene (Chen et al. 1996), which confirms that leptin is the satiety factor hypothesized by Coleman based on his parabiosis experiments. Binding of leptin to LepRb-bearing cells modulates a number of cellular signaling pathways, including phosphatidylinositol

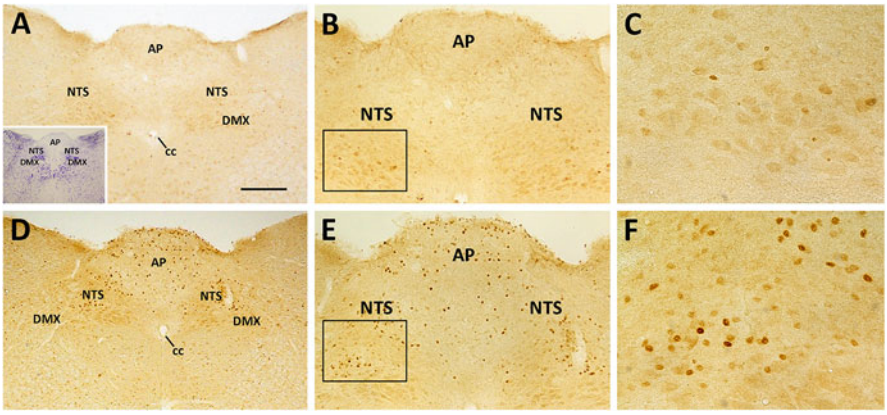

Fig. 2 c-Fos immunohistochemical expression in the DVC of the brainstem during fasting. In a normally-fed mouse (**a**–**c**), c-Fos immunoreactivity is almost undetectable. In a mouse fasted for 24 h (**d**–**f**), numerous c-Fos immunoreactive neurons appear in both the area postrema (AP) and the NTS. **c** and **f** are enlargements of the corresponding areas framed in **b** and **e**, respectively. cc, central canal. Bar: **a** and **d** 300 μm; inset of **a** 900 μm; **b** and **e**: 180 μm; **c** and **f** 35 μm

3-kinase, mammalian target of rapamycin, and AMP-dependent protein kinase. Activation of Janus kinase 2 (Jak2), leading to tyrosine phosphorylation, dimerization, and nuclear translocation of the signal transducer and activator of transcription 3 (STAT3), is the main signaling system activated by leptin and is required for accurate regulation of the energy balance (Villanueva and Mayers 2008). In situ hybridization and immunohistochemical studies have documented that LepRb is widely expressed in the hypothalamic and brainstem nuclei involved in energy balance regulation (Mercer et al. 1996; Schwartz et al. 1996; Fei et al. 1997; Elmquist et al. 1998). In experimental animals, intraperitoneal leptin injection induces a rapid increase in its blood levels, strongly activating the Jak2-STAT3 pathways in several neurons of the tuberal hypothalamic nuclei (Fig. 3) and the NTS of the brainstem (Fig. 4).

Studies aimed at characterizing the satiety action of leptin in the hypothalamus have stressed the crucial role of two populations of ARC neurons exerting opposite effects in feeding behavior: the neurons co-expressing neuropeptide Y (NPY) and agouti-related protein (AgRP) are found in the medial part of the ARC and are orexigenic, whereas the proopiomelanocortin (POMC) neurons are located in the lateral portion of the ARC and are anorexigenic (Aponte et al. 2011; Krashes et al. 2011; Zhan et al. 2013). Circulating leptin, reaching these neurons through the median eminence or the cerebrospinal fluid, or carried through the BBB by specific transporters, inhibits the orexigenic NPY/AgRP neurons and stimulates the anorexigenic POMC neurons (Kim et al. 2014). This well-characterized mechanism is followed by numerous and still poorly understood interactions involving leptin and neurotransmitter (dopamine, serotonin, and glutamate) and neuropeptidergic neuronal systems. “First order” leptin-responsive NPY/AgRP and POMC ARC

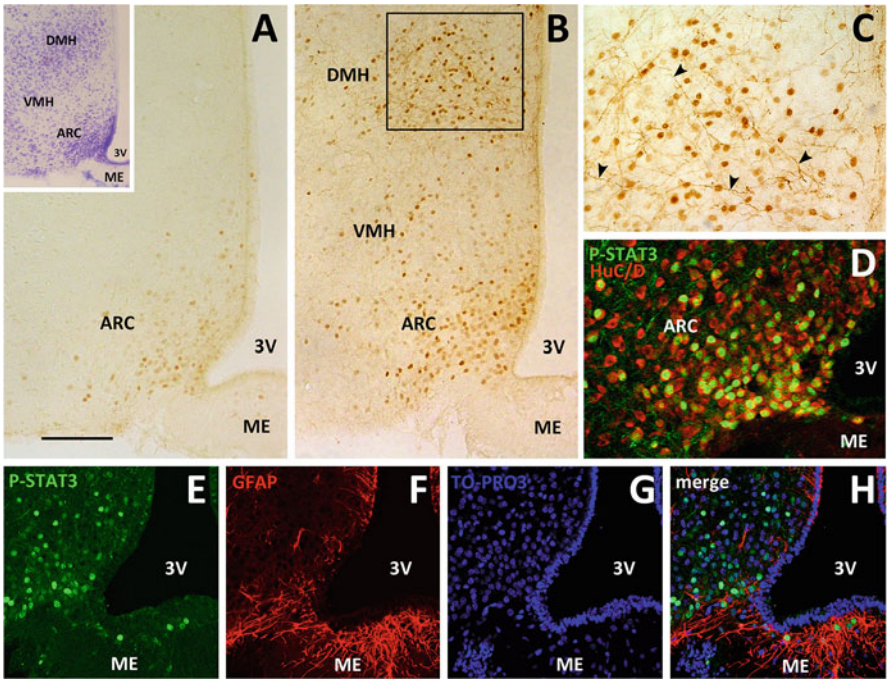

Fig. 3 Activation of the Jak2-STAT3 signaling pathway in the tuberal hypothalamus by circulating leptin. In a control mouse (**a**), phospo-STAT3 (P-STAT3) immunoreactivity is only slightly detectable in a few neurons of the medial part of the ARC. In a mouse intraperitoneally treated with leptin for 40 min (**b** and **c**), numerous P-STAT3 immunoreactive neurons appear in the ARC, the DMH and, to a lesser extent, in the VMH. C is the enlargement of the area framed in **b**, showing that also neuronal projections do express P-STAT3 (arrowheads) following leptin treatment. Double immunostaining experiments and confocal microscopy analyses in leptin-treated mice show that nuclear P-STAT3 immunoreactivity is located in neuronal cells (**d**), expressing the neuronal marker HuC/D, but not in the glial cells (**e**–**h**), visualized through the glial marker GFAP. TO-PRO3 is a fluorescent nuclear counterstaining. 3V, third ventricle; ME, median eminence. Bar: **a** and **b** 150 μm; inset of **a** 400 μm; **c** 60 μm; **d** 45 μm; **e**–**h** 100 μm

neurons project to "second order" neuronal populations located in medial structures of the tuberal hypothalamus (PVN, DMH, and VMH), where they stimulate anorexigenic peptidergic systems (e.g., brain-derived neurotrophic factor, BDNF), and in the lateral tuberal hypothalamus (perifornical area and LH), where they inhibit orexigenic peptidergic systems (e.g., orexin) (Morton et al. 2014). Importantly, the POMC neurons act on downstream neurons through melanocortin 3 and 4 receptors (MC4R) (Waterson and Horvath 2015). The medial structures are the "satiety center" and the lateral structures "the feeding center" of the stereotaxic approach. From the tuberal hypothalamus, the "satiety message" conveyed by circulating leptin spreads to other hypothalamic and extra-hypothalamic neurocircuits to induce the satiety sensation, halt eating, and promote energy-intensive responses and behaviors such as thermogenesis, immunity, locomotion, growth, and reproduction (Park and Ahima 2015).

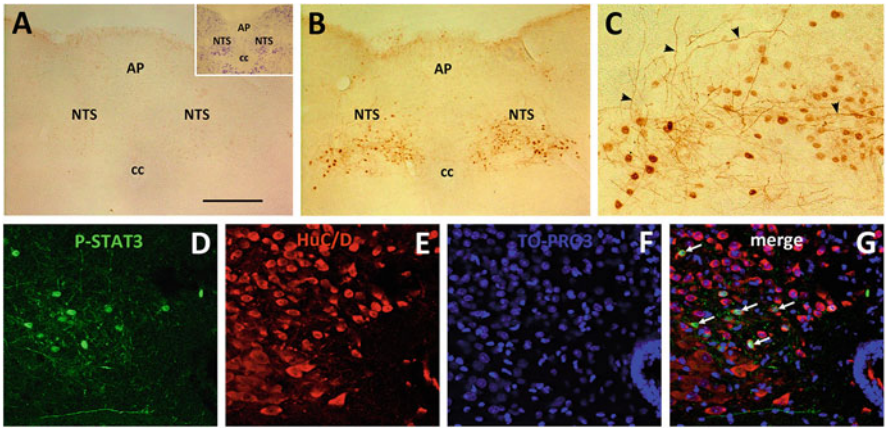

Fig. 4 Activation of the Jak2-STAT3 signaling pathway in the DVC of the brainstem by circulating leptin. In a control mouse (**a**), phospo-STAT3 (P-STAT3) immunoreactivity is not detectable in the DVC (see text for details). In a mouse intraperitoneally treated with leptin for 40 min (**b and c**), numerous P-STAT3 immunoreactive neurons appear in the NTS, where also neuronal projections are positive for P-STAT3 (arrowheads in **c**). Double immunostaining experiments and confocal microscopy analyses (**d**–**g**) show that P-STAT3 immunoreactivity in the NTS is located into neuronal cells expressing the neuronal marker HuC/D (arrows). TO-PRO3 is a fluorescent nuclear counterstaining. cc, central canal. Bar: **a** and **b** 150 μm; inset of **a** 400 μm; **c**–**g** 40 μm

The discovery of leptin, more than a decade ago, was hailed by the scientific community as the solution to the treatment of human obesity. Unfortunately, it soon emerged that, except for very rare leptin-deficient individuals (see below), obese humans are minimally responsive to exogenous leptin: they develop leptin resistance in the brain and even high levels of circulating leptin are unable to reduce feeding and body weight. Several mechanisms have been proposed to account for the leptin resistance seen in the obese, including defective leptin transport across the BBB (Banks 2003), impaired leptin intracellular signaling (Munzberg and Morrison 2015), and endoplasmic reticulum stress in leptin-sensitive neurons (Ozcan et al. 2009). The neurobiological basis of leptin resistance is a very active area of research with the potential to lead to the development of molecules that act as leptin sensitizers to treat obesity.

Insulin

Insulin produced and secreted by pancreatic beta cells regulates blood glucose and glucose metabolism by acting on peripheral organs. However, insulin also exerts effects on the brain, where it controls food intake and the energy balance. Glucose-induced insulin secretion is proportional to body fat stores (Bagdade et al. 1967), and circulating insulin enters the brain through a specific transport machinery (Baura et al. 1993). Intracerebroventricular administration of insulin reduces food intake in

experimental animals (Woods et al. 1979). Insulin receptors are diffusely expressed in the brain, especially in the hypothalamic and brainstem areas that are crucial for food intake regulation (Perry and Wang 2012). The anorectic effect of insulin is mainly due to inhibition of orexigenic NPY/AgRP neurons and stimulation of anorexigenic POMC neurons (Dodd and Tiganis 2017). Thus, its satiety action converges to a significant extent on the hypothalamic neurocircuits that are targeted by leptin, although its satiety action is less effective and involves different intracellular signaling systems. Notably, in the hypothalamus of morbidly obese patients, leptin resistance is often associated to insulin resistance.

Ghrelin

Ghrelin, first discovered as the endogenous ligand of the growth hormone secretagogue receptor 1a, is a peptide hormone produced and secreted into the blood by the stomach (Kojima et al. 1999). Only its acylated form is able to bind to ghrelin receptor in the brain and in peripheral organs and to cross the BBB (Kojima et al. 1999). Serum ghrelin concentrations are augmented by fasting and reduced by refeeding, and central or peripheral ghrelin administration strongly increases food intake, adiposity, and body weight in experimental animals (Tschöp et al. 2000; Nakazato et al. 2001) as well as humans, where it also enhances appetite (Wren et al. 2001). In the brain, ghrelin receptors are found in several hypothalamic and extrahypothalamic areas, such as hippocampus, substantia nigra, ventral tegmental area, and all three DVC components in the brainstem (Zigman et al. 2006). They are also highly abundant in NPY/AgRP neurons of the hypothalamic ARC, where selective re-expression of ghrelin receptor in fully ghrelin receptor-deficient knockout mice has been shown to restore the orexigenic response to administered ghrelin and to normalize the lowered blood glucose induced by caloric restriction (Wang et al. 2013). Based on these data, the orexigenic action of ghrelin is therefore held to be closely linked to the depolarization and activation of orexigenic NPY/AgRP neurons of the hypothalamic ARC. Overall, ghrelin stimulates eating and helps to maintain normal blood glucose levels upon fasting or calorie restriction. For this reasons, it is often referred to as the "hunger hormone." To date, it is the only known orexigenic hormone produced by the gastrointestinal tract. A role for ghrelin in feeding behavior has been documented by an action not only on the hypothalamus but also on the DVC of the brainstem (Suzuki et al. 2010). Furthermore, by interacting with several neurotransmitter and peptidergic systems of the brain, it also regulates complex energy-intensive processes and behaviors such as stress responses, growth, and reproduction (Al Massadi et al. 2017).

Cholecystokinin

CCK is a small peptide secreted from specific enteroendocrine cells of the duodenum, the first segment of the small intestine, and plays well-established roles in

digestive processes. The fatty and/or amino acids contained in the chyme entering the duodenum stimulate the release of CCK, which induces delivery into the small intestine of digestive enzymes from the pancreas and bile from the gallbladder. As noted above, in 1973 its circulating levels were found to increase rapidly in response to meals and it was demonstrated to act as a satiety factor (Gibbs et al. 1973). Subsequent studies confirmed its acute satiety effect also in humans (Kissileff et al. 1981; Beglinger et al. 2001). CCK receptors are widely distributed in the brain both in the tuberal hypothalamus and in the DVC of the brainstem (Ballaz 2017). However, attempts to correlate its blood levels to its anorectic effect have not been conclusive, CCK does not appear to be able to cross the BBB and, most importantly, central administration of CCK receptor antagonists does not blunt the satiety effect of peripherally administered CCK (Corp et al. 1997). The search for other ways by which CCK could act has led to the discovery that its satiety effect depends on a local and paracrine action on the gastrointestinal vagal sensory terminals that innervate the intestinal mucosa and project into the NTS. The anorectic effect of CCK is abolished by lesion of vagal afferent nerves by surgery (subdiaphragmatic vagotomy) or chemical treatment (using capsaicin, which selectively destroys small unmyelinated visceral sensory fibers) (Iwasaki and Yada 2012). Importantly, POMC neurons are not only found in the lateral part of the hypothalamic ARC, but also in the NTS of the brainstem, where a further POMC neuronal population is involved in energy balance regulation (Zhan et al. 2013). The CCK-sensitive vagal afferents activate NTS POMC neurons, which in turn recruit satiety brainstem neurocircuits through MC4R (Fan et al. 2004), thus mirroring the action of leptin and insulin in the ARC POMC system of the tuberal hypothalamus. Collectively, these studies show that the peripheral terminals of vagal afferents play an important role in energy balance regulation by sensing meal-evoked gut-derived peptides and acting on the DVC. Notably, a similar gut-to-brain satiety pathway has been hypothesized also for other gastrointestinal and pancreatic hormones that regulate feeding and metabolism, such as peptide YY and glucagon-like peptide-1 (GLP-1).

Peptide YY and Pancreatic Polypeptide

The NPY family of peptides comprises three highly homologous 36-amino acid peptides: NPY, peptide YY, and pancreatic polypeptide (PP). Whereas NPY is chiefly expressed by neuronal cells in the brain, peptide YY is primarily produced by enteroendocrine cells of the ileum and colonic mucosa, and PP by pancreatic islet PP cells (Ekblad and Sundler 2002). All these peptides act through at least five widely distributed functional receptors: Y1, Y2, Y4, Y5, and Y6. Nutrient ingestion stimulates gastrointestinal production and secretion in the blood of PP and peptide YY. A potential role for PP as a circulating satiety factor was first surmised based on the observation that meal-induced PP secretion was blunted in children with Prader-Willi syndrome, a rare childhood genetic disorder characterized by obesity, diabetes, cognitive impairment, and infertility (Zipf et al. 1981). Subsequent animal and

human studies confirmed its satiety effect. PP regulates food intake by acting on hypothalamic Y4 receptors, although its mechanism of action does not seem to involve primarily the ARC. It inhibits the orexin orexigenic pathway in the LH and simultaneously stimulates the BDNF anorexigenic pathway in VMH (Sainsbury et al. 2010).

Peptide YY is found in the blood in two forms, YY_{1-36} and YY_{3-36}, the latter form being the more effective (Chelikani et al. 2005). The mechanism of action of peptide YY_{3-36} is very different from that of PP. As described in Y2 receptor-deficient mice, where peripheral administration of neuropeptide YY_{3-36} evokes no anorectic response (Batterham et al. 2002), this satiety factor acts through Y2 receptor, not Y4 receptor. Y2 receptor is widespread in the body. In the hypothalamus, circulating neuropeptide YY_{3-36} may diffuse over the median eminence, reach the NPY/AgRP neurons of the ARC, and inhibit the electrical activity of the NPY orexigenic system by acting through Y2 receptors (Batterham et al. 2002). However, Y2 receptors are also found in peripheral vagal afferents, and bilateral subdiaphragmatic vagotomy reduces the anorectic effect of intraperitoneal neuropeptide YY_{3-36} (Abbott et al. 2005; Koda et al. 2005). These data indicate that neuropeptide YY_{3-36} acts both on hypothalamic ARC neurocircuits and on peripheral vagal afferents, where it likely evokes as yet unknown neural circuits that inhibit feeding behavior.

Glucagon-Like Peptide-1

The gut neuroendocrine cells that produce peptide YY also synthesize pre-proglucagon, a large precursor protein that is further processed to produce numerous biologically active peptides, including glucagon, GLP-1, GLP-2, and oxyntomodulin. All these peptides are secreted in the blood during feeding and play interconnected and redundant roles on digestive processes and metabolism, such as gastric emptying, gut motility, nutrient absorption, and insulin secretion (Spreckley and Murphy 2015). For many of them, a true action on the nervous system to reduce food intake is still debated. GLP-1 is the peptide that has attracted the most attention in the past few years, also because of its therapeutic potential for diabetes (see chapter ▶ "Roles of Gut Hormones in the Regulation of Food Intake and Body Weight"). It is an incretin hormone, whose primary effect is to enhance glucose-stimulated insulin release by pancreatic beta cells and to reduce blood sugar, but it also has satiety effects (Vilsbøll and Holst 2004). Gut vagal sensory terminals express GLP-1 receptors, whose stimulation evokes action potentials in vagal nodose ganglion neurons (Kakei et al. 2002). Importantly, the anorectic effect seen after peripheral administration of GLP-1 is abolished by abdominal vagotomy in rats (Abbot et al. 2005) and by capsaicin pretreatment in mice (Talsania et al. 2005). Altogether, these data suggest that the satiety effect of GLP-1 is to a large extent due to activation of the vagal-NTS brainstem route. However, GLP-1 receptors are also found in key brain areas for energy balance regulation, including hypothalamus and brainstem (Merchenthaler et al. 1999). Recently, circulating GLP-1 has been shown to exert an inhibitory effect on eating through direct activation of GLP-1 receptors in

the DVC of male rats (Punjabi et al. 2014), suggesting that the satiety effect of secreted GLP-1 is likely due to an action on both peripheral vagal terminals and brainstem feeding centers through as yet unknown mechanisms.

Amylin

Amylin, also known as islet amyloid polypeptide, is a 37-amino acid peptide co-secreted, together with insulin, by pancreatic beta cells in response to nutrient ingestion (Cooper et al. 1987). After its discovery, circulating amylin was shown to be involved in gastric emptying and in glucagon and digestive enzyme secretion (Hay et al. 2015). However, in rats food intake involves a rapid increase in blood amylin that correlates with meal size, and intraperitoneal administration of recombinant amylin reduces food intake in a dose-dependent manner (Lutz et al. 1995). In addition, intravenous administration of the amylin receptor antagonist AC187 stimulates eating in rats through increased meal size (Reidelberger et al. 2004). For these reasons, amylin is regarded as a physiological circulating satiety factor in both rodents and humans. Interestingly, its mechanism of action primarily involves the area postrema, the brainstem circumventricular organ. Amylin receptor is highly expressed in neurons of the area postrema (Becskei et al. 2004), and stereotactic injection of amylin in the area postrema inhibits eating, whereas injection of the amylin receptor antagonist AC187 stimulates it (Mollet et al. 2004). Finally, the satiety effect of amylin is abolished in animals with area postrema lesion, whereas it is maintained in capsaicin-treated rats and in animals subjected to subdiaphragmatic vagotomy (Lutz et al. 2001). The brainstem neurocircuits engaged by amylin have not yet been elucidated. However, in the area postrema, it activates a substantial population of noradrenergic neurons (Potes et al. 2010) that affect the excitability of NTS neurons, including POMC neurons, where the satiety message conveyed by amylin converges with that of other gastrointestinal satiety factors including CCK, peptide YY, and GLP-1.

Conclusion and Future Directions of Research

Our knowledge of the neuroendocrinology of the energy balance, gained in the past few decades, comes mainly from animal models. Albeit still largely incomplete, the evidence collected to date allows to make some general considerations on how hormones act on the brain to regulate feeding behavior, energy expenditure, and, ultimately, body weight.

Numerous hormones produced by peripheral organs affect energy balance regulation through such an action. Their metabolic information reaches the brain through at least four different routes: interaction with specific BBB transporters (insulin, leptin); diffusion to adjacent brain areas through the circumventricular organs (ghrelin); direct action on neurons in the circumventricular organs (amylin); and, finally, stimulation of peripheral vagal sensory afferents (CCK, peptide YY,

and GLP-1) and activation of gut-to-brain pathways. Importantly, these routes are not mutually exclusive and a hormone can often reach its brain targets through multiple routes. For instance, the area postrema is crucially involved in mediating the effect of amylin but also contains neurons and glial cells that sense several other circulating signals related to energy homeostasis, like ghrelin, CCK, and GLP-1 (Young 2012).

Each hormone involved in energy balance homeostasis likely regulates distinctive, subtly different features of feeding behavior, metabolism, and energy homeostasis. However, knowledge in this area is still very limited.

The vast majority of such hormones are satiety factors. Only one feeding factor, ghrelin, has been identified to date, possibly indicating that the brain's default setting is to search for food and to feed, and that circulating satiety cues keep the feeding neurocircuits inhibited and the satiety neurocircuits activated between meals.

Some energy balance hormones, including leptin, insulin, and ghrelin, seem to have a predominantly longer-term metabolic regulatory action, providing to the brain information on the body energy stores and playing a permissive or restrictive role on energy-intensive behaviors, such as growth and reproduction. These hormones primarily act on the neurocircuits of the tuberal hypothalamus. Other energy balance hormones, including CCK, neuropeptide YY, GLP-1, and amylin, appear to be more suited to playing a short-term action on satiety and feeding behavior and primarily act on brainstem feeding neurocircuits.

Emerging evidence indicates that mammalian energy balance hormones can affect feeding behavior through multiple parallel neurocircuits in different hypothalamic and brainstem areas involving diverse neurotransmitter and neuropeptidergic systems. At the same time, each neurocircuit is targeted by several energy balance hormones, giving rise to an extremely complex, overlapping, distributed, and redundant neuronal system. Thus, it is not surprising that, for instance, ghrelin knockout in adult mice affects neither feeding nor body weight (McFarlane et al. 2014) or that CCK-knockout mice exhibit a normal feeding behavior (Lo et al. 2008). From a physiological viewpoint, the redundancy may be explained with the need to ensure a normal or normal-like feeding behavior even in extreme environmental conditions, whereas from a pathological viewpoint it explains why it is very difficult to obtain significant and durable changes in body weight and to find effective drugs to treat human obesity.

However, basic neuroendocrinology research in this area has the potential to lead, in the near future, to the discovery of more effective anti-obesity drugs. Importantly, investigation of mutations homologous to those causing obesity in mouse models has allowed to identify some human monogenic obesity syndromes related to leptin deficiency (Farooqi et al. 1999) and dysfunctional mutations of *POMC* (Krude et al. 1998) or *MC4R* (Hinney et al. 1999) genes. Although these monogenic forms of obesity are rare, they indicate that the energy balance neurocircuits are evolutionarily highly conserved among species. Murine models are therefore suitable to study the central mechanisms of energy balance regulation and to identify novel molecular targets for the treatment of human obesity.

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