European Journal of Pharmacology 660 (2011) 2–12

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Review

Modulation of the central melanocortin system by leptin, insulin, and serotonin: Co-ordinated actions in a dispersed neuronal network

Kevin W. Williams [a], Michael M. Scott [a], Joel K. Elmquist [a,b,*]

[a] Division of Hypothalamic Research, Department of Internal Medicine, The University of Texas Southwestern Medical Center, Dallas, TX 75390 USA
[b] Department of Pharmacology, The University of Texas Southwestern Medical Center, Dallas, TX 75390 USA

ARTICLE INFO

Article history:
Received 2 September 2010
Received in revised form 8 October 2010
Accepted 2 November 2010
Available online 3 January 2011

Keywords:
Leptin
Insulin
Serotonin
Obesity
Diabetes
Patch-clamp

ABSTRACT

Over the past century, prevalent models of energy and glucose homeostasis have been developed from a better understanding of the neural circuits underlying obesity and diabetes. From the early hypothalamic lesion reports to the more recent pharmacological and molecular/genetic studies, the hypothalamic melanocortin system has been shown to play a critical role in the regulation of metabolism. This review attempts to highlight contributions to our current understanding of how numerous neuromodulators (leptin, insulin, and serotonin) integrate with the central melanocortin system to coordinate alterations in energy and glucose balance.

Published by Elsevier B.V.

Contents

1. Introduction

Obesity has reached epidemic proportions in the United States, with the majority of the population now overweight and greater than a quarter of the population classified as obese (Flegal et al., 2010). Alarmingly this is a trend which is not limited to the United States; rather obesity and its co-morbidities such as type II diabetes mellitus are on the rise and pose a serious threat to public health around the world (Shaw et al., 2010; Wild et al., 2004; Zimmet et al., 2001). To understand the causes and to develop treatments for obesity and type II diabetes mellitus, it is first necessary to unravel how numerous neuropeptides, neurotransmitters, receptors, and the central intracellular signaling pathways regulate coordinated energy and glucose homeostasis. Several studies have determined that the hypothalamus is a key component in the regulation of metabolic homeostasis, integrating information regarding the body's internal environment and orchestrating a series of coordinated endocrine, autonomic and

* Corresponding author. University of Texas Southwestern Medical Center at Dallas, 5323 Harry Hines Boulevard, Dallas, Texas 75390-9077. Tel.: +1 214 648 2911; fax: +1 214 648 5044.
E-mail address: Joel.Elmquist@UTSouthwestern.edu (J.K. Elmquist).

0014-2999/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.ejphar.2010.11.042

behavioral responses that maintain metabolic homeostasis. This review outlines the current understanding of leptin, insulin, and serotonin action, key regulators of glucose and energy homeostasis, largely within the hypothalamic melanocortin system.

2. Hypothalamic vs. pituitary obesity syndromes

The first clinical description of hypothalamic–pituitary injury resulting in obesity was reported in 1840 (Mohr, 1840). Bernhard Mohr described his patient, a 57 year old woman who experienced rapid weight gain, became obese within one year, and suffered from multiple neurological deficits, reviewed here (Brobeck, 1946). After a few years with her deteriorating condition she died and although post-mortem analysis revealed tumor-like degeneration of the pituitary body likely producing increased intracranial pressure upon adjacent parts of the brain little else was known about the causes of her condition. In 1900, Joseph Babinski noted a condition characterized by feminine obesity and sexual infantilism resulting from a tumor of the pituitary (Babinski, 1900). A year later, Alfred Fröhlich described a rare childhood metabolic disorder characterized by obesity, growth retardation, and retarded development of the genital organs also resultant of a pituitary tumor (Fröhlich, 1901). Since the reports of the Babinski–Fröhlich's syndrome, debate as to whether this disorder was due to pituitary insufficiency or hypothalamic damage ensued (Erdheim, 1904). Forty years later, A.W. Hetherington and Stephen Ranson performed a series of electrolytic lesions in the ventral medial hypothalamus of rats which replicated the obesity and hyperphagia previously observed in the Babinski–Fröhlich's syndrome (Hetherington and Ranson, 1940). Their work showed for the first time that the ventral medial hypothalamus, independent of any pituitary insufficiency, is required for proper energy homeostasis (Hetherington and Ranson, 1942). Collectively, these data highlight the necessity to understand the hypothalamic circuitry involved in the regulation of energy and glucose homeostasis.

3. The adipocyte-derived peptide leptin regulates energy and glucose balance

Claude Bernard first suggested the idea of homeostasis nearly 150 years ago when he introduced the concept of milieu intérieur. Numerous examples of homeostatic mechanisms exist in mammals, such as those that regulate energy and glucose homeostasis, largely relying on a feedback loop of a signal/hormone which regulates activities such as food intake, metabolic rate, glucose uptake/disposal or production. Gordon Kennedy is credited with establishing one homeostatic paradigm known as the “lipostatic hypothesis” — which states a signal released into the circulation in proportion to body adipose stores acts to regulate feeding and overall energy balance (Kennedy, 1953). A seminal study which extended the “lipostatic hypothesis” and helped to delineate a possible circulating factor involved in the regulation of energy balance was performed by Doug Coleman while working at Jackson Laboratories (Coleman, 1973; Coleman and Hummel, 1969). He studied mice in which two independent spontaneous autosomal recessive mutations occurred and resulted in obesity and diabetes; reviewed here (Williams et al., 2009). Briefly, Coleman's work identified a circulating factor which was absent in ob/ob mice that regulated energy and glucose homeostasis. Conversely, this circulating factor was made in excess in db/db mice yet these mice were resistant either because they lacked the receptor or the signaling cascade activated by this circulating factor was impaired. It wasn't until another 40 years had passed before the adipose-derived peptide leptin was first described and identified as the circulating factor in Coleman's studies which was required for proper energy and glucose balance (Zhang et al., 1994). Today, it is generally accepted that peripheral signals/hormones integrate with other factors such as the presence of food or social behavior to regulate food intake (Schwartz and Porte, 2005; Woods et al., 1998).

Shortly after leptin was discovered, leptin's cognate receptor was cloned (Tartaglia et al., 1995). The leptin receptor is a member of the type I cytokine receptor family and shares homology to glycoprotein 130, a transmembrane protein which forms a subunit of the interleukin-6 family of cytokines. Leptin has 6 identified receptors (LepRa-f), of which LepRb is defined as the longest isoform of the leptin receptor, containing the intracellular tyrosine residues necessary for activation of multiple intracellular signaling cascades (Chen et al., 1996; Lee et al., 1996; Myers et al., 2008; Takaya et al., 1996; Tartaglia et al., 1995). Upon ligand binding the leptin receptor (LepRb) dimerizes and triggers the activation of Janus activated kinases (JAKs) which phosphorylate the tyrosine residues on the cytoplasmic domain of the leptin receptor and thus initiating the multiple signaling cascades activated by leptin (Fig. 1). Peripheral leptin administration results in decreased food intake and body weight (Halaas et al., 1995). Interestingly intracerebroventricular leptin administration alone replicates the decrease in food intake and body weight observed after peripheral administration (Campfield et al., 1995; Halaas et al., 1997). Moreover, selective deletion of leptin receptors within the central nervous system (CNS) of mice recapitulates the db/db phenotype (Cohen et al., 2001). Together these data suggest that the CNS is a primary site of action for leptin to mediate effects of energy and glucose homeostasis. These data also led to the prevalent model of energy homeostasis in which leptin is not only an adipostatic secreted factor, but rather a metabolic cue that communicates directly to the brain the status of available energy stores (Schwartz and Porte, 2005). Importantly, leptin monotherapy was shown to correct a genetic deficiency of leptin in a small population of humans, supportive of a shared physiology from rodents to humans and suggesting that understanding leptin regulation of energy and glucose balance in rodents also advances an understanding of circuits involved in energy and glucose homeostasis in human physiology (Farooqi et al., 1999, 2002).

4. Discovery of insulin: physiological importance in the body and the brain

In 1869, Paul Langerhans was first to identify structures of unknown function throughout the pancreas resembling islands floating among acinar cells and later termed islets of Langerhans. Initially it was thought that the pancreas was solely critical for proper digestion, however at the turn of the century, several researchers were making critical observations which clearly established a link between the islets and diabetes mellitus. Joseph von Mering and Oskar Minkowski made the first major discovery in 1889 when they reported severe and fatal diabetes resulting from the complete removal of the pancreas from dogs (von Mering and Minkowski, 1889). Eugene Opie subsequently reported hyalinosis of the islets of Langerhans in patients suffering from diabetes mellitus suggesting degeneration of the islets contributed to the pathophysiology of the disease (Opie, 1900–1901a; b). Several attempts were made to identify what the islets were producing in an effort to treat diabetes; however it was not until the early 1920s when Frederick Banting and medical student Charles Best developed a method to isolate a viable extract from the pancreas of dogs while working in the lab space of John James Richard Macleod at the University of Toronto — reviewed here (Banting et al., 1922). The extract was found to contain isletin (later termed insulin) and injection of the extract into pancreatectomized dogs corrected diabetes (Banting and Best, 1922). In January 1922, after improvements in the quality and method of extraction, a Toronto teenager (L.T.) was the first successfully injected with Banting and Best's extract and almost completely corrected the signs of diabetes. Interestingly, it is estimated that the islets of Langerhans constitute less than 3% of the pancreas, yet their partial or

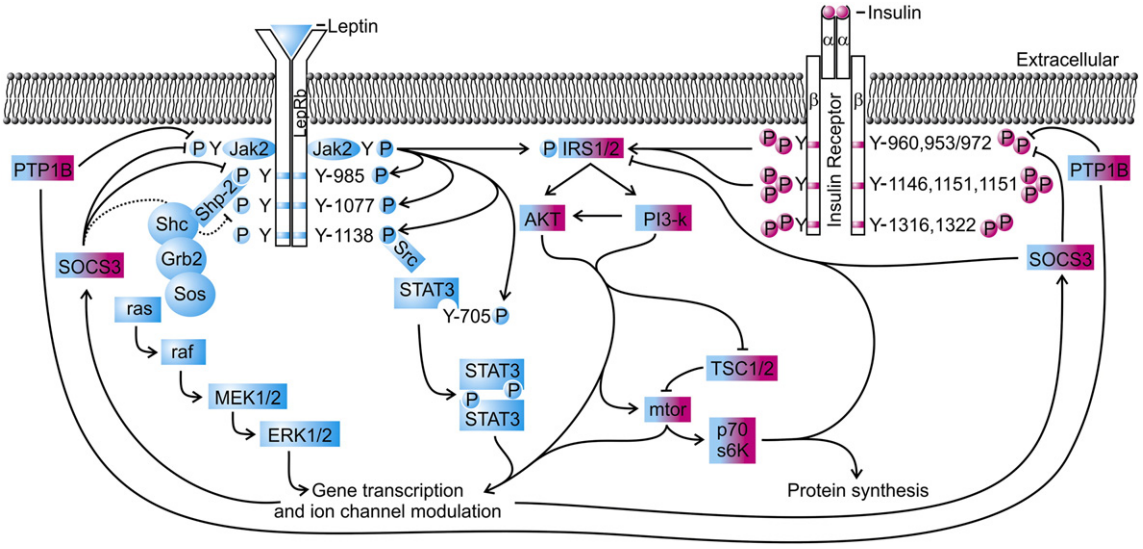

Fig. 1. Intracellular signaling by the leptin (LepRb — cyan) and insulin receptor (magenta). Leptin binding to its receptor exhibits simple kinetic properties suggesting that one molecule of leptin binds to the leptin receptor (left side). Leptin binding to the extracellular domain of LepRb dimer induces JAK2 tyrosine kinases, which are noncovalently associated proximally with the receptor, to autophosphorylate (Heldin, 1995; Ihle et al., 1994; Myers et al., 2008). Activated Jak2 subsequently phosphorylates a number of substrates including the tyrosine residues (Y985, 1077, and Y1138) on the intracellular domain of the leptin receptor. STAT3 is recruited via its SH2 (src homology) domain to the distal tyrosine residue (Y1138) and is then phosphorylated by JAK2 (Banks et al., 2000; Bates et al., 2003; White et al., 1997). STAT3 subsequently dimerizes and translocates to the nucleus to modify the transcription of several genes including SOCS3 (Banks et al., 2000; Bjorbaek et al., 1998). SOCS3 is a negative regulator of cytokine signaling and acts as a negative regulator of leptin in a feedback loop (Bjorbaek et al., 1998). SOCS3 inhibits phosphorylation of the proximal tyrosine residue (Y985) on the leptin receptor and to a lesser degree on tyrosine residue (Y1077) (Bjorbak et al., 2000; Eyckerman et al., 2000). SOCS3 can also inhibit JAK2 and STAT3 directly (Dunn et al., 2005; Sasaki et al., 2000; Sasaki et al., 1999). In addition to providing a binding site for SOCS3, the proximal tyrosine residue (Y985) also binds SHP2 upstream of the ERK/MAPK pathway (Bahrenberg et al., 2002; Banks et al., 2000; Bjorbak et al., 2000; Kloek et al., 2002). PTP-1B is largely associated with the surface of the ER and negatively regulates leptin signaling by dephosphorylating JAK2 (Zabolotny et al., 2002). JAK2 activates the IRS-PI3K pathway which has been shown to alter gene transcription and modify cellular activity via activation of conductances such as the Katp and TRPC channels (Hill et al., 2008; Mirshamsi et al., 2004; Niswender et al., 2001; Qiu et al., 2010; Spanswick et al., 1997). Leptin also activates the mammalian target of rapamycin-ribosomal S6 kinase (mtor-S6K) pathway which are kinases known to regulate transcription and protein synthesis (Blouet et al., 2008; Cota et al., 2008; Cota et al., 2006). In contrast to leptin, insulin binding to its receptor exhibits complex kinetic properties suggesting that one molecule of insulin may bind and activate the insulin receptor, however another molecule of insulin may bind to a lower affinity binding site on the receptor as well (right side). Insulin binding to the extracellular alpha subunits of the insulin receptor induces autophosporylation of the intracellular beta subunits and is critical for insulin action in vivo (White et al., 1984, 1988). The insulin receptor has 8 tyrosine residues which can be phosphorylated (Ullrich et al., 1985). These sites exist in 3 clusters: juxtamembrane (Y960 and Y950/972), tri-tyrosine (1146, 1150, and 1151), and c-terminus (1316 and 1322). The insulin receptor subsequently recruits and activates IRS proteins which in turn activate many of the same signaling cascades activated by leptin (cyan/magenta) including PI3K-AKT (Myers et al., 1992; Myers and White, 1993). The negative regulators of leptin receptor activity, SOCS3 and PTP1B, also feedback directly on the insulin receptor and inhibit signaling — both can also inhibit IRS1 directly (Elchebly et al., 1999; Emanuelli et al., 2001; Emanuelli et al., 2000; Goldstein et al., 2000; Kenner et al., 1996; Klaman et al., 2000). S6K also inhibits insulin signaling by phosphorylating IRS1 at multiple serine residues (Carlson et al., 2004; Harrington et al., 2004; Ozes et al., 2001; Tremblay et al., 2007).

entire destruction results in diabetes mellitus (Elayat et al., 1995; Orci et al., 1976; Overholser, 1925). Later research showed that the beta cells (β-cells) within pancreatic islets of Langerhans play a critical role in mammalian physiology by synthesizing and secreting insulin.

The insulin receptor is a transmembrane tyrosine kinase receptor retaining endogenous enzyme activity to transfer phosphate groups from ATP to tyrosine residues on the intracellular target proteins (Feener et al., 1993; Lee and Pilch, 1994; Ullrich et al., 1985). The insulin receptor contains two alpha and two beta subunits linked by disulfide bonds (Ullrich et al., 1985). The beta subunits span the plasma membrane and constitute the intracellular domain of the receptor, while the alpha subunits are entirely extracellular and retain the ligand binding site (Fig. 1). Insulin binding to the extracellular alpha subunits causes the beta subunits to autophosphorylate, thus activating various intracellular proteins/kinases which regulate biological activity (Lee and Pilch, 1994; Ullrich et al., 1985).

Diabetes mellitus, resulting from impaired insulin signaling, leads to several complications including coronary artery disease, retinopathy, stroke, kidney failure, and ultimately death. However, it is clear that proper maintenance of blood glucose levels can ameliorate these conditions. Fortunately, multiple therapies in combination with insulin exist today to treat diabetes and the metabolic perturbations that accompany this disease. Although less is known about the role of insulin in the brain, growing evidence suggests that insulin signaling in the brain is equally important as signaling in the periphery for the maintenance of glucose and energy homeostasis (Hill et al., 2010; Konner et al., 2007; Woods et al., 1979). For instance, similar to leptin receptors, insulin receptors are widely expressed in the CNS and maybe critical for neuronal survival (Havrankova et al., 1978a; Havrankova et al., 1978b). Moreover, Woods et al. (1979) showed that intracerebroventricular infusion of insulin reduced food intake and body weight. Mice lacking insulin receptors specifically in the CNS also displayed increased food intake, diet-induced obesity and mild insulin resistance (Bruning et al., 2000). Thus, increasing evidence suggests that in order to continue providing better therapeutics for the treatment of diabetes, we must fully understand the effects of insulin not only in the periphery but also in the brain. Given that leptin and insulin are both peripheral hormones which mediate effects on glucose and energy balance through circuits in the brain, the Elmquist laboratory and others has been focused on understanding the circuits which are required and/or sufficient for insulin and leptin action within the CNS.

5. Melanocortins: central regulators of energy and glucose homeostasis

The hypothalamic arcuate nucleus includes proopiomelanocortin (POMC) and neuropeptide-Y/agouti-related peptide (NPY/AgRP) neurons. POMC is a precursor polypeptide which undergoes post-translational processing to produce several biologically active

peptides called melanocortins (γ-melanocyte-stimulating hormone (MSH), adrenocorticotropic hormone (ACTH), β-lipotropin, α-MSH, corticotropin-like intermediate lobe peptide (CLIP), γ-lipotropin, β-endorphin, and β-MSH) (Chretien et al., 1979; Cochet et al., 1982; Nakanishi et al., 1979; Uhler and Herbert, 1983). Roger Cone and colleagues first cloned the melanocortin receptors in the 1990s (Adan and Gispen, 1997; Chhajlani et al., 1993; Chhajlani and Wikberg, 1992; Gantz et al., 1993a, b; Mountjoy et al., 1994; Mountjoy et al., 1992). There are 5 cognate melanocortin receptors (MC_{1-5} receptor) which are 7 transmembrane G-protein coupled receptors of the rhodopsin family. Several of the receptors have biologically active functions in the periphery such as regulating mammalian skin/hair color (MC_1 receptor) (Jackson, 1993; Robbins et al., 1993) stimulation of glucocorticoid production (MC_2 receptor) (Clark et al., 1993; Tsigos et al., 1993), and regulating the production of sebum in the exocrine glands (MC_5 receptor) (Griffon et al., 1994). However, MC_3 and MC_4 receptors are the only melanocortin receptors both highly expressed within the brain and involved in the regulation of energy balance. For instance, mice deficient for MC_3 receptors are characterized by modest increased weight gain and adiposity on a low-fat diet (Butler et al., 2000; Sutton et al., 2006). MC_4 receptor null mice on a low-fat diet show striking hyperphagia, obesity, hyperinsulinemia, and increased lean body and fat mass (Butler et al., 2001; Sutton et al., 2006). Exposure of MC_4 receptor null mice to a high-fat diet exacerbates the obesity and diabetes observed on a low-fat diet. Surprisingly, MC_3 receptor null mice on a high-fat diet share similar characteristics to high-fat diet fed MC_4 receptor null mice suggesting that MC_3 receptor null mice may have an important role in regulation peripheral metabolism and energy balance (Sutton et al., 2006). Interestingly, re-expression of MC_4 receptor in neurons of the paraventricular hypothalamus, a target of arcuate POMC and NPY/AgRP neurons, and a subpopulation of amygdala neurons, completely rescued the increased food intake and the majority (60%) of the obesity observed in MC_4 receptor null mice (Balthasar et al., 2005). These data highlight the importance of melanocortin signaling within the paraventricular hypothalamus as well as support a model which includes a distributed network of melanocortin neurons that regulate energy homeostasis. It should be noted that humans with mutations in the MC_4 receptor exhibit similar characteristics to MC_4 receptor null mice suggesting a critical role of MC_4 receptor in the regulation of energy balance in human physiology (Hinney et al., 1999; Vaisse et al., 1998; Yeo et al., 1998). Moreover, the work of O'Rahilly and colleagues showed that mutations in the MC_4 receptor gene are now recognized as the most common monogenic form of human obesity described to date (Barsh et al., 2000; Farooqi et al., 2003). Thus these data define a key role of melanocortin signaling in the regulation of energy and glucose homeostasis.

One of the melanocortins, α-MSH—an $MC_{3/4}$ receptor agonist, and its analogs potently inhibits food intake in the brain via activation of central melanocortin neurons (Fan et al., 1997; Ludwig et al., 1998; Rossi et al., 1998; Tsujii and Bray, 1989). Interestingly, an increase in serum leptin results in both an increase in POMC gene expression and the resulting peptide α-MSH (Mizuno et al., 1998; Schwartz et al., 1997; Thornton et al., 1997). Moreover, acute administration of leptin results in increased markers of cellular activation in POMC neurons (Elias et al., 1999). Furthermore, leptin administration in an acute slice preparation results in a leptin-induced depolarization of arcuate POMC neurons concomitant with activation of a putative mixed-cation conductance (Cowley et al., 2001; Hill et al., 2008; Qiu et al., 2010; Williams et al., 2010). A recent study extended these observations of the acute activation of POMC neurons by leptin suggesting the involvement of a TrpC channel in the leptin-induced depolarization (Qiu et al., 2010). However the subunits comprising the TrpC channel are currently unknown and future studies will undoubtedly delineate the properties of this leptin-activated mixed-cation conductance in arcuate POMC cells. Adjacent to arcuate melanocortin neurons are the NPY/AgRP cells. NPY has been associated with a number of physiological responses in the brain however a primary effect of NPY appears to be a reduction in food intake (Stanley and Leibowitz, 1985). In addition, AgRP colocalizes with NPY in arcuate neurons and functions as an inverse agonist to the melanocortin receptor (Chai et al., 2003; Hahn et al., 1998; Nijenhuis et al., 2001; Tota et al., 1999). Opposite to the actions of leptin in POMC neurons, leptin administration has been shown to inhibit gene transcription of NPY/AgRP in the hypothalamus (Schwartz et al., 1996a, b; Stephens et al., 1995). Leptin application also results in a decrease in GABAergic inhibitory post synaptic currents (IPSCs) onto POMC neurons resulting in a disinhibition of POMC cellular activity (Cowley et al., 2001). Due to previous observations which showed an inhibition of mediobasal hypothalamic neurons by leptin and a colocalization of GABA and NPY in synaptic boutons which synapse onto POMC neurons, it has largely been speculated that leptin hyperpolarizes and suppresses the cellular activity of NPY/AgRP neurons via a PI3K dependent activation of an ATP-sensitive potassium conductance (Cowley et al., 2001; Spanswick et al., 1997). Moreover, leptin application to rat NPY neurons, identified via single-cell PCR, resulted in a hyperpolarization which mirrored the previous reports on unidentified mediobasal hypothalamic neurons providing evidence for a leptin-induced inhibition of NPY/AgRP neurons (van den Top et al., 2004). Together, these data form the basic model of leptin regulation in NPY/AgRP and POMC neurons of the hypothalamic arcuate nucleus (Cone et al., 2001; Cowley et al., 2001). Also, these data are an indicator that the next era of obesity research has begun with the use of mouse genetics to test models of energy and glucose homeostasis.

6. The "Molecular Era" of obesity research

Identification and cloning of leptin, leptin receptors, and melanocortin receptors were key steps to the start of the "molecular era" of obesity research which incorporates the power of mouse genetics to test varying circuit models utilizing Cre-loxP (cyclization recombination-locus of X over P1) technology to perform genetic manipulation in a cell specific manner. Leptin receptors are widely expressed in the brain, however an enriched expression is observed within the mediobasal hypothalamus including the arcuate POMC neurons (Elias et al., 1999; Elmquist et al., 1998b). In a first attempt to tease apart leptin action on neural circuits in the brain a simple model was proposed. If the melanocortin POMC neurons are the primary site for mediating the effects of leptin to maintain energy homeostasis then deleting leptin receptors in POMC neurons alone should result in altered energy balance. Selective deletion of leptin receptors in POMC neurons resulted in a modest obesity in male and female mice (Balthasar et al., 2004). Interestingly, no effect was observed on food intake; rather the increased body weight was dependent on a decreased energy expenditure and locomotor activity (Fig. 2). Deletion of leptin receptors in POMC neurons also produced no or mild effects on glucose homeostasis (Balthasar et al., 2004; Shi et al., 2008). This was surprising given the fact that leptin deficient mice expressing a transgene of the POMC gene under the control of a neuron-specific promoter exhibited normalized glucose levels and insulin resistance (Mizuno et al., 2003). In subsequent studies consistent with these leptin data, selective deletion of suppressor of cytokine signaling 3 (SOCS3) in POMC cells resulted in enhanced leptin sensitivity and also improved glucose homeostasis on a chow diet while weight gain was reduced and insulin sensitivity and glucose homeostasis was improved on a high energy diet (Kievit et al., 2006). Importantly, another recent study examined the role of leptin receptors in adjacent AgRP neurons (van de Wall et al., 2008). Van de Wall and colleagues found that selective deletion from AgRP neurons also resulted in hyperphagia, decreased locomotor activity, and modest obesity (Fig. 2), supporting the basic model of leptin action postulated from electrophysiological data described in the prior section. These data

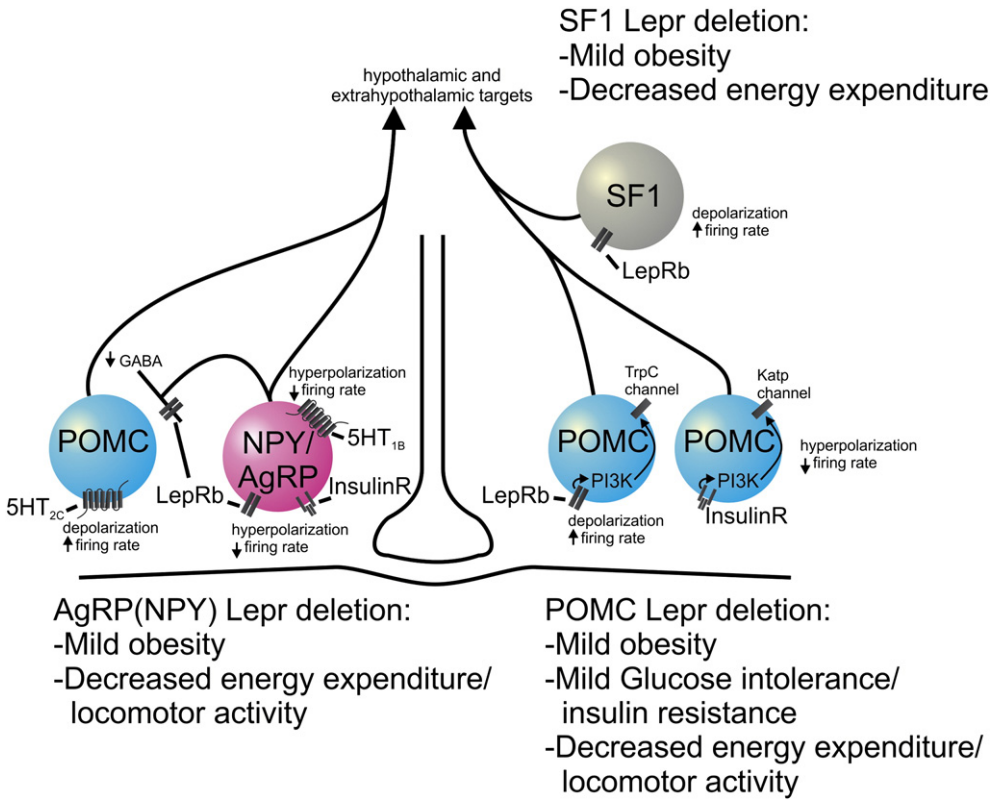

Fig. 2. Effects of leptin receptor deletion in distributed hypothalamic neurons. The effects of direct leptin action in POMC (cyan), NPY/AgRP (magenta), or SF1 (grey) cells on energy and glucose homeostasis are revealed by selective deletion of leptin receptors. Mice lacking leptin receptors in POMC neurons alone exhibit decreased energy expenditure and locomotor activity resulting in increased adiposity and concomitant mild diabetes. Similarly, deletion of leptin receptors in SF1 neurons results in decreased energy expenditure resulting in increased adiposity. Finally, lack of leptin signaling in NPY/AgRP neurons results in mild obesity from decreased energy expenditure and locomotor activity; however glucose and insulin sensitivity was unaffected.
Fig. modified from (Williams et al., 2009).

also suggest another cell population within the arcuate other than NPY/AgRP neurons contributes to the leptin-induced regulation of glucose balance and additional studies were needed in order to determine the role of melanocortin neurons in glucose homeostasis.

The Elmquist, Lowell and Chua laboratories further examined the role of leptin signaling in the arcuate nucleus to regulate glucose balance by generating a mouse homozygous for a FLPe-reactivatable, leptin receptor null allele [Lepr(neo/neo)] (Coppari et al., 2005). The Lepr(neo/neo) mice were similar to the db/db mouse in that they are hyperphagic, obese, hyperglycemic, hyperinsulinemic, infertile, and hypoactive. Unilateral injection of an adeno-associated virus expressing FLPe-recombinase into the arcuate nucleus of Lepr(neo/neo) mice resulted in a modest decrease in food intake and bodyweight. Unilateral reactivation of Leprs in the arcuate nucleus restored deficits in glucose homeostasis such that hyperinsulinemia was improved and blood glucose levels were normalized demonstrating the sufficiency of arcuate leptin signaling in maintaining glucose homeostasis. Similar results were found when leptin receptors were expressed via adenoviral injection into the arcuate nucleus of Koletsky rats (fa^k/fa^k) which are deficient for the leptin receptor and recent evidence by the same group suggests a leptin-induced hypothalamic regulation of hepatic insulin sensitivity is dependent upon the hepatic vagal branch (German et al., 2009; Morton et al., 2005, 2003). Christain Bjorbaek's group recently investigated whether overexpression of LepRb in POMC cells alone was sufficient to regulate energy and glucose homeostasis (Huo et al., 2009). They found that leptin receptor expression in POMC neurons of a db/db mouse resulted in a decreased food intake and energy expenditure concomitant with a modest reduction in body weight. Importantly, overexpression of leptin receptors in POMC neurons alone normalized the high blood glucose levels observed in the db/db mouse. Together these data suggest that direct leptin action in POMC neurons is required for the maintenance of energy homeostasis through energy expenditure and locomotor regulation and is not required but maybe sufficient for the maintenance of proper glucose homeostasis (Fig. 2). Intriguingly, a recent report suggests that leptin alone can normalize blood glucose levels of type I diabetics and keep the blood glucose levels in the normal range for extended periods of time possibly via regulation of the peripheral hormone glucagon (Yu et al., 2008). It remains to be determined if direct leptin action in POMC neurons may underlie the dramatic effect of leptin to ameliorate insulin insensitivity in diabetes mellitus.

7. Distributed networks: model for the central regulation of energy and glucose homeostasis

The relatively modest effects observed on body weight in mice lacking leptin receptors in POMC neurons suggested that the effects of leptin require a distributed network of neurons. In support of multiple sites of leptin action, selective deletion of leptin receptors in adjacent ventral medial hypothalamic steroidogenic factor-1 (SF-1) neurons resulted in an obesity which mirrored that observed in the POMC-LepR null mouse (Bingham et al., 2008; Dhillon et al., 2006). Moreover, deletion of leptin receptors in POMC and SF1 neurons resulted in an additive effect on body weight. The leptin-induced acute activation of SF1 neurons was similar to the acute effects of leptin observed in POMC neurons such that most SF1 positive neurons were depolarized resulting in an increased firing rate in response to leptin — an effect not observed in SF1-LepR null mice (Fig. 2). However, future studies are needed to assess whether these data support a similar leptin-induced activation of a putative mixed-cation conductance in SF1 cells. Interestingly,

SF1-LepR null mice fed a high-fat diet gained considerably more weight than their wildtype counterpart. The gain in body weight of SF1-LepR null mice on a HFD was also greater than predicted by leptin deficiency or HFD alone. Together, these data suggest that direct leptin action within the ventral medial hypothalamus plays an important role in energy homeostasis through modifying energy expenditure and notably protects against HFD-induced obesity possibly by regulation of diet-induced thermogenesis (Fig. 2). It should be noted that Heatherington and Ranson's electrolytic lesion studies in the 1940s were largely inclusive of the ventral medial hypothalamus. Therefore these data are essentially a modern (21st century) correlate of the Heatherington and Ranson lesion studies and support a requirement of the ventral medial hypothalamus for proper energy balance. These data also highlight growing evidence for a distributed network of leptin responsive neurons within the hypothalamus contributing to the central actions of leptin to regulate energy and glucose homeostasis.

Balthasar et al. (2005), reported that expression of MC_4 receptors in the PVN and amygdala is responsible for the reduction in food intake mediated by melanocortin signaling and not for the effects on metabolism. Moreover, local and long-term antagonism of melanocortin signaling in the paraventricular hypothalamus, dorsal medial hypothalamus, and lateral hypothalamus, by use of an adeno-associated virus expressing Agouti under the control of the cytomegalovirus promoter, differentially induced hyperphagia and accelerated body weight gain without apparent changes in central neuropeptide systems that are known to stimulate food intake (Kas et al., 2004). Thus, melanocortin action may be seen as being distributed, across numerous CNS nuclei. While a significant amount of research has focused on the hypothalamic actions of both leptin and the melanocortins, projections from hypothalamic melanocortin neurons to the hindbrain have also been implicated in the modulation of energy homeostasis. Similar to the hypothalamus, the melanocortins act in concert with leptin to affect both food intake and metabolic rate, through actions in the nucleus of the solitary tract, area postrema and vagus nerve. Recent work involving the injection of the $MC_{3/4}$ receptor agonist MTII directly into the NTS at relatively low concentrations resulted in an increased metabolic and cardiac rate with higher doses of MTII producing a reduction in food intake and acute body weight loss (Skibicka and Grill, 2009b). Use of the $MC_{3/4}$ receptor antagonist SHU, injected into the NTS, has been demonstrated to antagonize the effect of leptin injected into the arcuate nucleus, blocking the leptin dependent reduction in meal size and frequency (Zheng et al., in press). Interestingly, leptin may also be modulating food intake and metabolic rate through a direct action on neurons of the hindbrain. In particular, hindbrain delivery of leptin results in tachycardia and hyperthermia coupled with a reduction in food intake that is blocked by hindbrain application of the $MC_{3/4}$ receptor antagonist SHU (Grill et al., 2002; Skibicka and Grill, 2009a). The mechanism of leptin action in the hindbrain, specifically in the NTS, has been suggested to involve a reduction in food intake that results from changes in meal size that results from the potentiation of the vagal gastric distention signal (Emond et al., 2001; Grill et al., 2002; Huo et al., 2007; Schwartz and Moran, 2002). Recent work by Roger Cone and colleagues suggests CCK activates POMC neurons of the NTS and results in a reduction in food intake, an effect which also requires MC_4 receptor activation downstream of POMC neurons in the hindbrain (Fan et al., 2004). Furthermore, leptin potentiates the NTS response to a gastric preload and to the satiation signal CCK, enhancing the food intake reducing actions of these stimuli. In agreement with this observation, neurons of the dorsal vagal complex have been shown to be directly responsive to leptin, resulting in changes in acute neuronal activity (Williams and Smith, 2006; Williams et al., 2007). Finally, knock down of leptin receptor expression recapitulated the results obtained from hindbrain leptin injection, producing hyperphagia for both chow and high fat diet coupled with a decreased sensitivity to CCK resulting in increased weight gain (Hayes et al., 2010). Collectively, these data describing leptin and melanocortin action in the hindbrain, highlight the distributed nature of satiation signaling within the CNS.

8. The role of melanocortin PI3K in energy balance

Multiple signaling cascades are activated by leptin with each arm contributing to different facets of energy and glucose homeostasis (Fig. 1). There are several reports supportive of a role for one such signaling cascade, phosphoinositide 3-kinase (PI3K), in the effects of leptin and insulin action within the arcuate. For example, Barsh and colleagues utilized a fluorescent reporter for PI3K activity targeted to AgRP or POMC neurons (Wanting Xu et al., 2005). They found that leptin and insulin both triggered membrane accumulation of the PI3K fluorescent reporter which could be blocked by pretreatment with PI3K inhibitors. Additionally, a recent report showed that insulin increased phosphatidylinositol (3,4,5)-triphosphate (PIP3) levels in the arcuate nucleus of mice including POMC neurons — an effect absent in mice that the PIP3 phosphatase, PTEN, was disrupted selectively in POMC cells (Plum et al., 2006). Moreover, a subpopulation of neurons in the mediobasal hypothalamus is hyperpolarized by both leptin and insulin, an effect dependent upon a PI3K induced-activation of an ATP-sensitive potassium channel (Spanswick et al., 1997; Spanswick et al., 2000). Leptin and insulin both increase the phosphorylation of Akt and other PI3K signaling intermediates in the arcuate nucleus (Mirshamsi et al., 2004). Importantly, peripheral or central administration of leptin or insulin also activates IRS-2 associated PI3K in the hypothalamus of rats and PI3K inhibitors block the ability of icv leptin or insulin to reduce food intake (Niswender et al., 2003; Niswender et al., 2001). Together, these data suggest that the weight reducing effects of leptin and insulin depend on the PI3K activity within the hypothalamus and leptin and insulin act in parallel to stimulate PI3K activity in POMC neurons. Thus we investigated the role of class IA PI3K in leptin and insulin action on POMC neurons in a cell-type specific manner (Hill et al., 2008). We used a model in which a mouse was globally deficient for the P85β regulatory subunit and the P85α regulatory subunit was selectively deleted from POMC neurons in a Cre-dependent manner. These animals differed from those with a selective STAT3 deletion in POMC cells such that animals which lacked PI3K in POMC neurons had normal body weights food intake and glucose levels (Xu et al., 2007). However these animals failed to suppress food intake in response to acute leptin administration. Moreover, leptin failed to modify the cellular activity of POMC neurons lacking PI3K regulatory subunits. These data are supportive of the concept that leptin signaling is comprised of multiple cascades constituting the metabolic effects of leptin action. This study was also the first to delineate the requirement of PI3K signaling in POMC neurons for the acute effects of insulin on arcuate POMC neurons. Interestingly insulin and leptin both modify arcuate cellular activity via PI3K signaling, however leptin depolarizes while insulin hyperpolarizes POMC cells via a putative mixed cation channel and a Katp conductance, respectively. At first glance, it may seem illogical that leptin activates while insulin inhibits arcuate POMC neurons via the same signaling cascade, PI3K. However it was unclear whether the acute effects of leptin and insulin are observed in similar or distinct populations of POMC neurons.

We next assessed how leptin and insulin may acutely interact within POMC cells of the mediobasal hypothalamus by combining histological techniques with patch-clamp electrophysiology (Williams et al., 2010). We found that leptin and insulin receptors appeared to be distributed in an exclusive rostro-caudal and medio-lateral patterns. Additionally, the acute effects of leptin and insulin mirrored these anatomical patterns. Moreover, all POMC neurons which were acutely inhibited by insulin failed to show the characteristic leptin-induced depolarization. In all these data suggest a segregation of the acute effects of leptin and insulin within arcuate POMC cells (Fig. 2). However, a recent report suggested

that both acute leptin and insulin responses can be observed in a small subset of arcuate POMC cells suggestive that this segregation may not be absolute (Al-Qassab et al., 2009). Al-Qassab and colleagues also found that the effects of leptin and insulin in POMC and NPY/AgRP neurons maybe dependent upon the various catalytic isoforms of Class Ia PI3K. For instance, 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. Moreover, the weight reducing effects of central leptin administration was absent in POMC P110β null mice. Accordingly, the leptin-induced depolarization of POMC cells requires P110β, while P110α and P110β are both required to mediate the insulin-induced hyperpolarization in POMC cells. Similarly, P110β appeared more important in AgRP neurons for the regulation of energy balance such that selective deletion of P110β resulted in hypophagia, leanness, and resistance to diet-induced obesity. AgRP P110β null mice also experienced an increased sensitivity to the weight reducing effects of centrally administered leptin. Interestingly, both P110α and P110β are required for the acute effects of insulin on AgRP cells. However, contrary to the prevailing basic model of the acute effects of leptin and insulin in the arcuate nucleus the authors failed to observe a leptin-induced hyperpolarization of NPY/AgRP cells, and instead reported an insulin-induced depolarization of AgRP cells (Cone et al., 2001; Cowley et al., 2001; van den Top et al., 2004). Moreover, deletion of P110α or P110β or pharmacological inhibition of PI3K in NPY/AgRP cells revealed an insulin-induced hyperpolarization which was dependent upon a Katp channel. Clearly further investigation is needed in order to delineate the disparity of acute effects on cellular activity between the current study and previous work (Al-Qassab et al., 2009; Cowley et al., 2001; van den Top et al., 2004). However together these data are supportive of a segregation of leptin and insulin responses within POMC neurons and is intriguing given the variety of downstream targets of POMC cells and the differing metabolic phenotypes observed from selective deletion of either leptin or insulin receptor in POMC cells (Baker and Herkenham, 1995; Elias et al., 1999, 1998; Elmquist et al., 1998a; Swanson and Kuypers, 1980; Zheng et al., 2005).

9. Functional redundancy of leptin and insulin action in melanocortin neurons

Deletion of leptin receptors in POMC neurons results in moderate obesity without any effect on glucose homeostasis (Balthasar et al., 2004). Deletion of insulin receptors alone from POMC neurons fails to influence energy or glucose homeostasis (Konner et al., 2007). We recently reported that leptin and insulin alter the cellular activity in disparate subgroups of arcuate POMC cells, however these data did not address the potential crosstalk of leptin and insulin signaling within POMC neurons as it relates to signal transduction pathways which regulate genomic transcription and may ultimately regulate energy and glucose homeostasis (Williams et al., 2010). Thus we designed experiments to assess the requirement of insulin and leptin receptors to regulate energy and glucose balance within POMC neurons (Hill et al., 2010). Interestingly deletion of both insulin and leptin receptors in POMC neurons resulted in modest effects on body weight with more profound effects on glucose balance inducing hepatic insulin resistance and severe diabetes not seen in either single deletion alone. The double deletion also resulted in reduced fertility and elevated androgen levels in female mice in pattern reminiscent of polycystic ovarian syndrome (PCOS). Thus the double knockout unmasks a liver phenotype as well as a role of leptin/insulin signaling in POMC neurons to regulate fertility. Moreover, these data suggest that POMC neurons can regulate glucose and insulin independent of body weight. In support of divergent actions on body weight and glucose homeostasis Roger Unger and colleagues recently showed that a single injection of leptin normalizes the deranged homeostasis of rodents with type 1 diabetes, an effect that may be dependent on leptin signaling in POMC neurons (Yu et al., 2008).

10. Serotonin promotes proper energy balance via arcuate melanocortin neurons

Similar to prior leptin and insulin studies, genetic mouse models have been used to describe a role for the serotonergic (5HT) system in the regulation of feeding behavior and body weight. Serotonin has been shown to have profound effects on energy homeostasis (Nonogaki et al., 1998; Tecott et al., 1995). This has led to the development of therapeutics, such as sibutramine and D-fenfluramine (D-Fen), which target the serotoninergic system in order to achieve reductions in body weight by reducing food intake and increasing energy expenditure (Bray et al., 1999; Rowland and Carlton, 1986). Serotoninergic terminals converge on the arcuate nucleus which is supportive of an endogenous role of serotonin in regulating POMC and NPY neurons (Kiss et al., 1984; Zhou et al., 2005). Lora Heisler and colleagues found that similar to leptin action in the arcuate nucleus, serotonin activates POMC neurons while inhibiting NPY neurons (Heisler et al., 2002, 2006). Moreover, the serotonin induced activation of POMC neurons is thought to be mediated by the $5HT_{2C}R$, while the inhibition of NPY neurons may be dependent on the $5HT_{1B}R$ (Heisler et al., 2002, 2006). The similarity between leptin and serotonin regulation of arcuate neuronal activity suggests that serotonin induced-regulation of melanocortin neurons may be at least partly responsible for the serotonin induced effects on food intake and energy expenditure. In support of this idea, mice with a global $5HT_{2C}R$ deficiency are both hyperphagic and obese (Nonogaki et al., 1998; Tecott et al., 1995). Interestingly, D-Fen's anorexigenic effects are dependent upon $5HT_{2C}R$ activation (Vickers et al., 1999). Heisler and colleagues also demonstrated that the reduction in food intake requires MC_4 receptors downstream of arcuate POMC neurons (Heisler et al., 2002, 2006; Lam et al., 2008). Clearly, serotonin can modify the activity of arcuate melanocortin neurons, however the involvement of $5HT_{2C}Rs$ in the regulation of POMC neurons and the sufficiency of serotonin signaling in POMC neurons to regulate energy homeostasis was unclear. In an attempt to determine the sufficiency of serotonin signaling in POMC neurons we generated a mouse model that is globally deficient for $5HT_{2C}R$ expression by inserting a loxP-flanked transcription blocker (loxTB) into the $5HT_{2C}R$ gene (2C null) (Xu et al., 2008). We then mated these 2C null mice to POMC-Cre mice which resulted in $5HT_{2C}R$ reactivation selectively in POMC neurons (2C/POMC). Selective expression of $5HT_{2C}Rs$ in POMC neurons normalized the hyperphagia, hyperactivity, obesity, and attenuated pharmacological responses to 5-HT therapeutics observed in $5HT_{2C}R$ nulls. These data suggest that expression of $5HT_{2C}Rs$ selectively in POMC neurons is sufficient to normalize defects in food intake and weight gain observed in 2C null mice and suggests an important role of serotonergic signaling in POMC neurons to maintain energy homeostasis. In addition to obesity and hyperphagia, mice lacking $5HT_{2C}R$ develop hyperinsulinemia and impaired glucose tolerance (Nonogaki et al., 1998). Moreover, $5HT_{2C}R$ agonists improve glucose sensitivity and insulin resistance in a model of DIO (Zhou et al., 2007). Zhou and colleagues also showed that similar to the effects of $5HT_{2C}R$ agonists on food intake and weight gain, $5HT_{2C}R$ agonists require MC_4 receptors for improvement in glucose homeostasis. Interestingly, mice deficient for both $5HT_{2C}R$ and leptin (OB2C) exhibited an additive effect on impaired glucose tolerance suggesting a synergistic action of leptin and serotonin to regulate glucose homeostasis (Wade et al., 2008). Future experiments will have to delineate if the $5HT2_{2C}R$ induced improvements in diabetes are dependent upon central melanocortin action. Also, as previously discussed, recent evidence in our laboratory suggests that the acute effects of leptin on POMC neurons are distinct from the acute effects of insulin. It is currently unclear whether the effects of serotonin on arcuate POMC neurons

will segregate to one of these populations or whether serotonin may be acting on an entirely separate subpopulation of POMC neurons, ultimately adding another level of complexity to our understanding of leptin, insulin, and serotonin action within arcuate POMC neurons. Thus, although much progress has been made in understanding the role of serotonin in regulating energy and glucose homeostasis, many questions remain and await further investigation.

11. Summary

In summary, since the seminal observations linking obesity with hypothalamic dysfunction, much progress has been made in understanding the neural circuitry controlling metabolic homeostasis. Numerous hormones, including insulin and leptin along with neuronal signals such as serotonin and the melanocortins have been shown to act on specific neuronal populations to modulate food intake, glucose homeostasis and energy expenditure. Within the medial basal hypothalamus, POMC and NPY/AGRP neurons have been identified as important intergrators of metabolically relevant neuronal and hormonal inputs which act antagonistically to influence energy balance. Through the use of a molecular genetic approach, neuron specific manipulation of leptin, insulin, serotonin and melanocortin receptors has revealed the necessity and sufficiency of metabolic signaling in the maintenance of homeostasis in specific nuclei not only within the hypothalamus and the POMC–AGRP/NPY circuit but in other structures including the hindbrain. Both receptor deletion and reactivation studies have subsequently described a distributed network of neurons that responds to these salient metabolic cues. Lastly, further studies investigating the mechanism of action of these cues have revealed specific cascades, such as signaling through PI3K, responsible for select actions of leptin and insulin in both the arcuate and ventral medial hypothalamic nuclei of the hypothalamus. Thus, while considerable work remains to be done, our understanding of the functional neuroanatomy and physiology of the networks controlling metabolic homeostasis has advanced considerably, presenting us with novel targets for the development of therapeutic interventions targeted for the treatment of diabetes and obesity.

Acknowledgments

This work was supported by the National Institutes of Health (K01 DK087780 to KWW, K99 DA024719 to MMS, and DK53301, MH61583, DK081185 and DK71320 to JKE) and by an ADA Smith Family Foundation Pinnacle Program Award to JKE.

References

Adan, R.A., Gispen, W.H., 1997. Brain melanocortin receptors: from cloning to function. Peptides 18, 1279–1287.

Al-Qassab, H., Smith, M.A., Irvine, E.E., Guillermet-Guibert, J., Claret, M., Choudhury, A.I., Selman, C., Piipari, K., Clements, M., Lingard, S., Chandarana, K., Bell, J.D., Barsh, G.S., Smith, A.J., Batterham, R.L., Ashford, M.L., Vanhaesebroeck, B., Withers, D.J., 2009. Dominant role of the p110beta isoform of PI3K over p110alpha in energy homeostasis regulation by POMC and AgRP neurons. Cell Metab. 10, 343–354.

Babinski, J.F.F., 1900. Tumeur du corps pituitaire sans acromégalie et avec arrét de développement des organes génitaux. Rev. Neurol. 8, 531–533.

Bahrenberg, G., Behrmann, I., Barthel, A., Hekerman, P., Heinrich, P.C., Joost, H.G., Becker, W., 2002. Identification of the critical sequence elements in the cytoplasmic domain of leptin receptor isoforms required for Janus kinase/signal transducer and activator of transcription activation by receptor heterodimers. Mol. Endocrinol. 16, 859–872.

Baker, R.A., Herkenham, M., 1995. Arcuate nucleus neurons that project to the hypothalamic paraventricular nucleus: neuropeptidergic identity and consequences of adrenalectomy on mRNA levels in the rat. J. Comp. Neurol. 358, 518–530.

Balthasar, N., Coppari, R., McMinn, J., Liu, S.M., Lee, C.E., Tang, V., Kenny, C.D., McGovern, R.A., Chua Jr., S.C., Elmquist, J.K., Lowell, B.B., 2004. Leptin receptor signaling in POMC neurons is required for normal body weight homeostasis. Neuron 42, 983–991.

Balthasar, N., Dalgaard, L.T., Lee, C.E., Yu, J., Funahashi, H., Williams, T., Ferreira, M., Tang, V., McGovern, R.A., Kenny, C.D., Christiansen, L.M., Edelstein, E., Choi, B., Boss, O., Aschkenasi, C., Zhang, C.Y., Mountjoy, K., Kishi, T., Elmquist, J.K., Lowell, B.B., 2005. Divergence of melanocortin pathways in the control of food intake and energy expenditure. Cell 123, 493–505.

Banks, A.S., Davis, S.M., Bates, S.H., Myers Jr., M.G., 2000. Activation of downstream signals by the long form of the leptin receptor. J. Biol. Chem. 275, 14563–14572.

Banting, F.G., Best, C.H., 1922. Pancreatic extracts. J. Lab. Clin. Med. 115, 254–272.

Banting, F.G., Best, C.H., Collip, J.B., Campbell, W.R., Fletcher, A.A., 1922. Pancreatic extracts in the treatment of diabetes mellitus. Can. Med. Assoc. J. 12, 141–146.

Barsh, G.S., Farooqi, I.S., O'Rahilly, S., 2000. Genetics of body-weight regulation. Nature 404, 644–651.

Bates, S.H., Stearns, W.H., Dundon, T.A., Schubert, M., Tso, A.W., Wang, Y., Banks, A.S., Lavery, H.J., Haq, A.K., Maratos-Flier, E., Neel, B.G., Schwartz, M.W., Myers Jr., M.G., 2003. STAT3 signalling is required for leptin regulation of energy balance but not reproduction. Nature 421, 856–859.

Bingham, N.C., Anderson, K.K., Reuter, A.L., Stallings, N.R., Parker, K.L., 2008. Selective loss of leptin receptors in the ventromedial hypothalamic nucleus results in increased adiposity and a metabolic syndrome. Endocrinology 149, 2138–2148.

Bjorbaek, C., Elmquist, J.K., Frantz, J.D., Shoelson, S.E., Flier, J.S., 1998. Identification of SOCS-3 as a potential mediator of central leptin resistance. Mol. Cell 1, 619–625.

Bjorbak, C., Lavery, H.J., Bates, S.H., Olson, R.K., Davis, S.M., Flier, J.S., Myers Jr., M.G., 2000. SOCS3 mediates feedback inhibition of the leptin receptor via Tyr985. J. Biol. Chem. 275, 40649–40657.

Blouet, C., Ono, H., Schwartz, G.J., 2008. Mediobasal hypothalamic p70 S6 kinase 1 modulates the control of energy homeostasis. Cell Metab. 8, 459–467.

Bray, G.A., Blackburn, G.L., Ferguson, J.M., Greenway, F.L., Jain, A.K., Mendel, C.M., Mendels, J., Ryan, D.H., Schwartz, S.L., Scheinbaum, M.L., Seaton, T.B., 1999. Sibutramine produces dose-related weight loss. Obes. Res. 7, 189–198.

Brobeck, J.R., 1946. Mechanism of the development of obesity in animals with hypothalamic lesions. Physiol. Rev. 26, 541–559.

Bruning, J.C., Gautam, D., Burks, D.J., Gillette, J., Schubert, M., Orban, P.C., Klein, R., Krone, W., Muller-Wieland, D., Kahn, C.R., 2000. Role of brain insulin receptor in control of body weight and reproduction. Sci. NY N. Y 289, 2122–2125.

Butler, A.A., Kesterson, R.A., Khong, K., Cullen, M.J., Pelleymounter, M.A., Dekoning, J., Baetscher, M., Cone, R.D., 2000. A unique metabolic syndrome causes obesity in the melanocortin-3 receptor-deficient mouse. Endocrinology 141, 3518–3521.

Butler, A.A., Marks, D.L., Fan, W., Kuhn, C.M., Bartolome, M., Cone, R.D., 2001. Melanocortin-4 receptor is required for acute homeostatic responses to increased dietary fat. Nat. Neurosci. 4, 605–611.

Campfield, L.A., Smith, F.J., Guisez, Y., Devos, R., Burn, P., 1995. Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. Sci. NY N. Y 269, 546–549.

Carlson, C.J., White, M.F., Rondinone, C.M., 2004. Mammalian target of rapamycin regulates IRS-1 serine 307 phosphorylation. Biochem. Biophys. Res. Commun. 316, 533–539.

Chai, B.X., Neubig, R.R., Millhauser, G.L., Thompson, D.A., Jackson, P.J., Barsh, G.S., Dickinson, C.J., Li, J.Y., Lai, Y.M., Gantz, I., 2003. Inverse agonist activity of agouti and agouti-related protein. Peptides 24, 603–609.

Chen, H., Charlat, O., Tartaglia, L.A., Woolf, E.A., Weng, X., Ellis, S.J., Lakey, N.D., Culpepper, J., Moore, K.J., Breitbart, R.E., Duyk, G.M., Tepper, R.I., Morgenstern, J.P., 1996. Evidence that the diabetes gene encodes the leptin receptor: identification of a mutation in the leptin receptor gene in db/db mice. Cell 84, 491–495.

Chhajlani, V., Wikberg, J.E., 1992. Molecular cloning and expression of the human melanocyte stimulating hormone receptor cDNA. FEBS Lett. 309, 417–420.

Chhajlani, V., Muceniece, R., Wikberg, J.E., 1993. Molecular cloning of a novel human melanocortin receptor. Biochem. Biophys. Res. Commun. 195, 866–873.

Chretien, M., Benjannet, S., Gossard, F., Gianoulakis, C., Crine, P., Lis, M., Seidah, N.G., 1979. From beta-lipotropin to beta-endorphin and 'pro-opio-melanocortin'. Can. J. Biochem. 57, 1111–1121.

Clark, A.J., McLoughlin, L., Grossman, A., 1993. Familial glucocorticoid deficiency associated with point mutation in the adrenocorticotropin receptor. Lancet 341, 461–462.

Cochet, M., Chang, A.C., Cohen, S.N., 1982. Characterization of the structural gene and putative 5′-regulatory sequences for human proopiomelanocortin. Nature 297, 335–339.

Cohen, P., Zhao, C., Cai, X., Montez, J.M., Rohani, S.C., Feinstein, P., Mombaerts, P., Friedman, J.M., 2001. Selective deletion of leptin receptor in neurons leads to obesity. J. Clin. Investig. 108, 1113–1121.

Coleman, D.L., 1973. Effects of parabiosis of obese with diabetes and normal mice. Diabetologia 9, 294–298.

Coleman, D.L., Hummel, K.P., 1969. Effects of parabiosis of normal with genetically diabetic mice. Am. J. Physiol. 217, 1298–1304.

Cone, R.D., Cowley, M.A., Butler, A.A., Fan, W., Marks, D.L., Low, M.J., 2001. The arcuate nucleus as a conduit for diverse signals relevant to energy homeostasis. Int. J. Obes. Relat. Metab. Disord. 25 (Suppl 5), S63–S67.

Coppari, R., Ichinose, M., Lee, C.E., Pullen, A.E., Kenny, C.D., McGovern, R.A., Tang, V., Liu, S.M., Ludwig, T., Chua, S.C., Lowell, B.B., Elmquist, J.K., 2005. The hypothalamic arcuate nucleus: A key site for mediating leptin's effects on glucose homeostasis and locomotor activity. Cell Metab. 1, 63–72.

Cota, D., Proulx, K., Smith, K.A., Kozma, S.C., Thomas, G., Woods, S.C., Seeley, R.J., 2006. Hypothalamic mTOR signaling regulates food intake. Sci. NY N. Y 312, 927–930.

Cota, D., Matter, E.K., Woods, S.C., Seeley, R.J., 2008. The role of hypothalamic mammalian target of rapamycin complex 1 signaling in diet-induced obesity. J. Neurosci. 28, 7202–7208.

Cowley, M.A., Smart, J.L., Rubinstein, M., Cerdan, M.G., Diano, S., Horvath, T.L., Cone, R.D., Low, M.J., 2001. Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus. Nature 411, 480–484.

Dhillon, H., Zigman, J.M., Ye, C., Lee, C.E., McGovern, R.A., Tang, V., Kenny, C.D., Christiansen, L.M., White, R.D., Edelstein, E.A., Coppari, R., Balthasar, N., Cowley, M.A., Chua Jr., S., Elmquist, J.K., Lowell, B.B., 2006. Leptin directly activates SF1 neurons in the VMH, and this action by leptin is required for normal body-weight homeostasis. Neuron 49, 191–203.

Dunn, S.L., Bjornholm, M., Bates, S.H., Chen, Z., Seifert, M., Myers Jr., M.G., 2005. Feedback inhibition of leptin receptor/Jak2 signaling via Tyr1138 of the leptin receptor and suppressor of cytokine signaling 3. Mol. Endocrinol. 19, 925–938.

Elayat, A.A., el-Naggar, M.M., Tahir, M., 1995. An immunocytochemical and morphometric study of the rat pancreatic islets. J. Anat. 186 (Pt 3), 629–637.

Elchebly, M., Payette, P., Michaliszyn, E., Cromlish, W., Collins, S., Loy, A.L., Normandin, D., Cheng, A., Himms-Hagen, J., Chan, C.C., Ramachandran, C., Gresser, M.J., Tremblay, M.L., Kennedy, B.P., 1999. Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene. Sci. NY N. Y 283, 1544–1548.

Elias, C.F., Lee, C., Kelly, J., Aschkenasi, C., Ahima, R.S., Couceyro, P.R., Kuhar, M.J., Saper, C.B., Elmquist, J.K., 1998. Leptin activates hypothalamic CART neurons projecting to the spinal cord. Neuron 21, 1375–1385.

Elias, C.F., Aschkenasi, C., Lee, C., Kelly, J., Ahima, R.S., Bjorbaek, C., Flier, J.S., Saper, C.B., Elmquist, J.K., 1999. Leptin differentially regulates NPY and POMC neurons projecting to the lateral hypothalamic area. Neuron 23, 775–786.

Elmquist, J.K., Ahima, R.S., Elias, C.F., Flier, J.S., Saper, C.B., 1998a. Leptin activates distinct projections from the dorsomedial and ventromedial hypothalamic nuclei. Proc. Natl Acad. Sci. USA 95, 741–746.

Elmquist, J.K., Bjorbaek, C., Ahima, R.S., Flier, J.S., Saper, C.B., 1998b. Distributions of leptin receptor mRNA isoforms in the rat brain. J. Comp. Neurol. 395, 535–547.

Emanuelli, B., Peraldi, P., Filloux, C., Sawka-Verhelle, D., Hilton, D., Van Obberghen, E., 2000. SOCS-3 is an insulin-induced negative regulator of insulin signaling. J. Biol. Chem. 275, 15985–15991.

Emanuelli, B., Peraldi, P., Filloux, C., Chavey, C., Freidinger, K., Hilton, D.J., Hotamisligil, G.S., Van Obberghen, E., 2001. SOCS-3 inhibits insulin signaling and is up-regulated in response to tumor necrosis factor-alpha in the adipose tissue of obese mice. J. Biol. Chem. 276, 47944–47949.

Emond, M., Ladenheim, E.E., Schwartz, G.J., Moran, T.H., 2001. Leptin amplifies the feeding inhibition and neural activation arising from a gastric nutrient preload. Physiol. Behav. 72, 123–128.

Erdheim, J., 1904. Über hypophysengangsgeschwulste und hirmcholesteatome. Sitzungsb Kais Akad Wissen Math Naturw Klin 113, 537.

Eyckerman, S., Broekaert, D., Verhee, A., Vandekerckhove, J., Tavernier, J., 2000. Identification of the Y985 and Y1077 motifs as SOCS3 recruitment sites in the murine leptin receptor. FEBS Lett. 486, 33–37.

Fan, W., Boston, B.A., Kesterson, R.A., Hruby, V.J., Cone, R.D., 1997. Role of melanocortinergic neurons in feeding and the agouti obesity syndrome. Nature 385, 165–168.

Fan, W., Ellacott, K.L., Halatchev, I.G., Takahashi, K., Yu, P., Cone, R.D., 2004. Cholecystokinin-mediated suppression of feeding involves the brainstem melanocortin system. Nat. Neurosci. 7, 335–336.

Farooqi, I.S., Jebb, S.A., Langmack, G., Lawrence, E., Cheetham, C.H., Prentice, A.M., Hughes, I.A., McCamish, M.A., O'Rahilly, S., 1999. Effects of recombinant leptin therapy in a child with congenital leptin deficiency. N. Engl. J. Med. 341, 879–884.

Farooqi, I.S., Matarese, G., Lord, G.M., Keogh, J.M., Lawrence, E., Agwu, C., Sanna, V., Jebb, S.A., Perna, F., Fontana, S., Lechler, R.I., DePaoli, A.M., O'Rahilly, S., 2002. Beneficial effects of leptin on obesity, T cell hyporesponsiveness, and neuroendocrine/metabolic dysfunction of human congenital leptin deficiency. J. Clin. Investig. 110, 1093–1103.

Farooqi, I.S., Keogh, J.M., Yeo, G.S., Lank, E.J., Cheetham, T., O'Rahilly, S., 2003. Clinical spectrum of obesity and mutations in the melanocortin 4 receptor gene. N. Engl. J. Med. 348, 1085–1095.

Feener, E.P., Backer, J.M., King, G.L., Wilden, P.A., Sun, X.J., Kahn, C.R., White, M.F., 1993. Insulin stimulates serine and tyrosine phosphorylation in the juxtamembrane region of the insulin receptor. J. Biol. Chem. 268, 11256–11264.

Flegal, K.M., Carroll, M.D., Ogden, C.L., Curtin, L.R., 2010. Prevalence and trends in obesity among US adults, 1999–2008. JAMA 303, 235–241.

Fröhlich, A., 1901. Ein fall von tumor der hypophysis cerebri ohne akromegalie. Wien. klinische Rundsch. 15 (833–836), 906–908.

Gantz, I., Konda, Y., Tashiro, T., Shimoto, Y., Miwa, H., Munzert, G., Watson, S.J., DelValle, J., Yamada, T., 1993a. Molecular cloning of a novel melanocortin receptor. J. Biol. Chem. 268, 8246–8250.

Gantz, I., Miwa, H., Konda, Y., Shimoto, Y., Tashiro, T., Watson, S.J., DelValle, J., Yamada, T., 1993b. Molecular cloning, expression, and gene localization of a fourth melanocortin receptor. J. Biol. Chem. 268, 15174–15179.

German, J., Kim, F., Schwartz, G.J., Havel, P.J., Rhodes, C.J., Schwartz, M.W., Morton, G.J., 2009. Hypothalamic leptin signaling regulates hepatic insulin sensitivity via a neurocircuit involving the vagus nerve. Endocrinology 150, 4502–4511.

Goldstein, B.J., Bittner-Kowalczyk, A., White, M.F., Harbeck, M., 2000. Tyrosine dephosphorylation and deactivation of insulin receptor substrate-1 by protein-tyrosine phosphatase 1B. Possible facilitation by the formation of a ternary complex with the Grb2 adaptor protein. J. Biol. Chem. 275, 4283–4289.

Griffon, N., Mignon, V., Facchinetti, P., Diaz, J., Schwartz, J.C., Sokoloff, P., 1994. Molecular cloning and characterization of the rat fifth melanocortin receptor. Biochem. Biophys. Res. Commun. 200, 1007–1014.

Grill, H.J., Schwartz, M.W., Kaplan, J.M., Foxhall, J.S., Breininger, J., Baskin, D.G., 2002. Evidence that the caudal brainstem is a target for the inhibitory effect of leptin on food intake. Endocrinology 143, 239–246.

Hahn, T.M., Breininger, J.F., Baskin, D.G., Schwartz, M.W., 1998. Coexpression of Agrp and NPY in fasting-activated hypothalamic neurons. Nat. Neurosci. 1, 271–272.

Halaas, J.L., Gajiwala, K.S., Maffei, M., Cohen, S.L., Chait, B.T., Rabinowitz, D., Lallone, R.L., Burley, S.K., Friedman, J.M., 1995. Weight-reducing effects of the plasma protein encoded by the obese gene. Sci. NY N. Y 269, 543–546.

Halaas, J.L., Boozer, C., Blair-West, J., Fidahusein, N., Denton, D.A., Friedman, J.M., 1997. Physiological response to long-term peripheral and central leptin infusion in lean and obese mice. Proc. Natl Acad. Sci. USA 94, 8878–8883.

Harrington, L.S., Findlay, G.M., Gray, A., Tolkacheva, T., Wigfield, S., Rebholz, H., Barnett, J., Leslie, N.R., Cheng, S., Shepherd, P.R., Gout, I., Downes, C.P., Lamb, R.F., 2004. The TSC1-2 tumor suppressor controls insulin-PI3K signaling via regulation of IRS proteins. J. Cell Biol. 166, 213–223.

Havrankova, J., Roth, J., Brownstein, M., 1978a. Insulin receptors are widely distributed in the central nervous system of the rat. Nature 272, 827–829.

Havrankova, J., Schmechel, D., Roth, J., Brownstein, M., 1978b. Identification of insulin in rat brain. Proc. Natl Acad. Sci. USA 75, 5737–5741.

Hayes, M.R., Skibicka, K.P., Leichner, T.M., Guarnieri, D.J., DiLeone, R.J., Bence, K.K., Grill, H.J., 2010. Endogenous leptin signaling in the caudal nucleus tractus solitarius and area postrema is required for energy balance regulation. Cell Metab 11, 77-83

Heisler, L.K., Cowley, M.A., Tecott, L.H., Fan, W., Low, M.J., Smart, J.L., Rubinstein, M., Tatro, J.B., Marcus, J.N., Holstege, H., Lee, C.E., Cone, R.D., Elmquist, J.K., 2002. Activation of central melanocortin pathways by fenfluramine. Sci. NY N. Y 297, 609–611.

Heisler, L.K., Jobst, E.E., Sutton, G.M., Zhou, L., Borok, E., Thornton-Jones, Z., Liu, H.Y., Zigman, J.M., Balthasar, N., Kishi, T., Lee, C.E., Aschkenasi, C.J., Zhang, C.Y., Yu, J., Boss, O., Mountjoy, K.G., Clifton, P.G., Lowell, B.B., Friedman, J.M., Horvath, T., Butler, A.A., Elmquist, J.K., Cowley, M.A., 2006. Serotonin reciprocally regulates melanocortin neurons to modulate food intake. Neuron 51, 239–249.

Heldin, C.H., 1995. Dimerization of cell surface receptors in signal transduction. Cell 80, 213–223.

Hetherington, A.W., Ranson, S.W., 1940. Hypothalamic lesions and adiposity in the rat. Anat. Rec. 78, 149–172.

Hetherington, A.W., Ranson, S.W., 1942. Effect of early hypophysectomy on hypothalamic obesity. Endocrinology 31, 30–34.

Hill, J.W., Williams, K.W., Ye, C., Luo, J., Balthasar, N., Coppari, R., Cowley, M.A., Cantley, L.C., Lowell, B.B., Elmquist, J.K., 2008. Acute effects of leptin require PI3K signaling in hypothalamic proopiomelanocortin neurons in mice. J. Clin. Investig. 118, 1796–1805.

Hill, J.W., Elias, C.F., Fukuda, M., Williams, K.W., Berglund, E.D., Holland, W.L., Cho, Y.R., Chuang, J.C., Xu, Y., Choi, M., Lauzon, D., Lee, C.E., Coppari, R., Richardson, J.A., Zigman, J.M., Chua, S., Scherer, P.E., Lowell, B.B., Bruning, J.C., Elmquist, J.K., 2010. Direct insulin and leptin action on pro-opiomelanocortin neurons is required for normal glucose homeostasis and fertility. Cell Metab. 11, 286–297.

Hinney, A., Schmidt, A., Nottebom, K., Heibult, O., Becker, I., Ziegler, A., Gerber, G., Sina, M., Gorg, T., Mayer, H., Siegfried, W., Fichter, M., Remschmidt, H., Hebebrand, J., 1999. Several mutations in the melanocortin-4 receptor gene including a nonsense and a frameshift mutation associated with dominantly inherited obesity in humans. J. Clin. Endocrinol. Metab. 84, 1483–1486.

Huo, L., Maeng, L., Bjorbaek, C., Grill, H.J., 2007. Leptin and the control of food intake: neurons in the nucleus of the solitary tract are activated by both gastric distension and leptin. Endocrinology 148, 2189–2197.

Huo, L., Gamber, K., Greeley, S., Silva, J., Huntoon, N., Leng, X.H., Bjorbaek, C., 2009. Leptin-dependent control of glucose balance and locomotor activity by POMC neurons. Cell Metab. 9, 537–547.

Ihle, J.N., Witthuhn, B.A., Quelle, F.W., Yamamoto, K., Thierfelder, W.E., Kreider, B., Silvennoinen, O., 1994. Signaling by the cytokine receptor superfamily: JAKs and STATs. Trends Biochem. Sci. 19, 222–227.

Jackson, I.J., 1993. Molecular genetics. Colour-coded switches. Nature 362, 587–588.

Kas, M.J., Tiesjema, B., van Dijk, G., Garner, K.M., Barsh, G.S., ter Brake, O., Verhaagen, J., Adan, R.A., 2004. Induction of brain-region-specific forms of obesity by agouti. J. Neurosci. 24, 10176–10181.

Kennedy, G.C., 1953. The role of depot fat in the hypothalamic control of food intake in the rat. Proceedings of the Royal Society of London. Series B. Containing papers Biol. character 140, 578–596.

Kenner, K.A., Anyanwu, E., Olefsky, J.M., Kusari, J., 1996. Protein-tyrosine phosphatase 1B is a negative regulator of insulin- and insulin-like growth factor-I-stimulated signaling. J. Biol. Chem. 271, 19810–19816.

Kievit, P., Howard, J.K., Badman, M.K., Balthasar, N., Coppari, R., Mori, H., Lee, C.E., Elmquist, J.K., Yoshimura, A., Flier, J.S., 2006. Enhanced leptin sensitivity and improved glucose homeostasis in mice lacking suppressor of cytokine signaling-3 in POMC-expressing cells. Cell Metab. 4, 123–132.

Kiss, J., Leranth, C., Halasz, B., 1984. Serotoninergic endings on VIP-neurons in the suprachiasmatic nucleus and on ACTH-neurons in the arcuate nucleus of the rat hypothalamus. A combination of high resolution autoradiography and electron microscopic immunocytochemistry. Neurosci. Lett. 44, 119–124.

Klaman, L.D., Boss, O., Peroni, O.D., Kim, J.K., Martino, J.L., Zabolotny, J.M., Moghal, N., Lubkin, M., Kim, Y.B., Sharpe, A.H., Stricker-Krongrad, A., Shulman, G.I., Neel, B.G., Kahn, B.B., 2000. Increased energy expenditure, decreased adiposity, and tissue-specific insulin sensitivity in protein-tyrosine phosphatase 1B-deficient mice. Mol. Cell. Biol. 20, 5479–5489.

Kloek, C., Haq, A.K., Dunn, S.L., Lavery, H.J., Banks, A.S., Myers Jr., M.G., 2002. Regulation of Jak kinases by intracellular leptin receptor sequences. J. Biol. Chem. 277, 41547–41555.

Konner, A.C., Janoschek, R., Plum, L., Jordan, S.D., Rother, E., Ma, X., Xu, C., Enriori, P., Hampel, B., Barsh, G.S., Kahn, C.R., Cowley, M.A., Ashcroft, F.M., Bruning, J.C., 2007. Insulin action in AgRP-expressing neurons is required for suppression of hepatic glucose production. Cell Metab. 5, 438–449.

Lam, D.D., Przydzial, M.J., Ridley, S.H., Yeo, G.S., Rochford, J.J., O'Rahilly, S., Heisler, L.K., 2008. Serotonin 5-HT2C receptor agonist promotes hypophagia via downstream activation of melanocortin 4 receptors. Endocrinology 149, 1323–1328.

Lee, J., Pilch, P.F., 1994. The insulin receptor: structure, function, and signaling. Am. J. Physiol. 266, C319–C334.
Lee, G.H., Proenca, R., Montez, J.M., Carroll, K.M., Darvishzadeh, J.G., Lee, J.I., Friedman, J.M., 1996. Abnormal splicing of the leptin receptor in diabetic mice. Nature 379, 632–635.
Ludwig, D.S., Mountjoy, K.G., Tatro, J.B., Gillette, J.A., Frederich, R.C., Flier, J.S., Maratos-Flier, E., 1998. Melanin-concentrating hormone: a functional melanocortin antagonist in the hypothalamus. Am. J. Physiol. 274, E627–E633.
Mirshamsi, S., Laidlaw, H.A., Ning, K., Anderson, E., Burgess, L.A., Gray, A., Sutherland, C., Ashford, M.L., 2004. Leptin and insulin stimulation of signalling pathways in arcuate nucleus neurones: PI3K dependent actin reorganization and KATP channel activation. BMC Neurosci. 5, 54.
Mizuno, T.M., Kleopoulos, S.P., Bergen, H.T., Roberts, J.L., Priest, C.A., Mobbs, C.V., 1998. Hypothalamic pro-opiomelanocortin mRNA is reduced by fasting and [corrected] in ob/ob and db/db mice, but is stimulated by leptin. Diabetes 47, 294–297.
Mizuno, T.M., Kelley, K.A., Pasinetti, G.M., Roberts, J.L., Mobbs, C.V., 2003. Transgenic neuronal expression of proopiomelanocortin attenuates hyperphagic response to fasting and reverses metabolic impairments in leptin-deficient obese mice. Diabetes 52, 2675–2683.
Mohr, B., 1840. Hypertrophie der hypophysis cerebri und dadurchbedingter druck auf die hirngrundflache, insbesndere auf die Schnerven, das chiasma deselben und den linkseitigen. Hirnschenkel Wschr. Ges. Heilk 6, 565–571.
Morton, G.J., Niswender, K.D., Rhodes, C.J., Myers Jr., M.G., Blevins, J.E., Baskin, D.G., Schwartz, M.W., 2003. Arcuate nucleus-specific leptin receptor gene therapy attenuates the obesity phenotype of Koletsky (fa(k)/fa(k)) rats. Endocrinology 144, 2016–2024.
Morton, G.J., Gelling, R.W., Niswender, K.D., Morrison, C.D., Rhodes, C.J., Schwartz, M.W., 2005. Leptin regulates insulin sensitivity via phosphatidylinositol-3-OH kinase signaling in mediobasal hypothalamic neurons. Cell Metab. 2, 411–420.
Mountjoy, K.G., Robbins, L.S., Mortrud, M.T., Cone, R.D., 1992. The cloning of a family of genes that encode the melanocortin receptors. Sci. NY N. Y 257, 1248–1251.
Mountjoy, K.G., Mortrud, M.T., Low, M.J., Simerly, R.B., Cone, R.D., 1994. Localization of the melanocortin-4 receptor (MC4-R) in neuroendocrine and autonomic control circuits in the brain. Mol. Endocrinol. 8, 1298–1308.
Myers Jr., M.G., White, M.F., 1993. The new elements of insulin signaling. Insulin receptor substrate-1 and proteins with SH2 domains. Diabetes 42, 643–650.
Myers Jr., M.G., Backer, J.M., Sun, X.J., Shoelson, S., Hu, P., Schlessinger, J., Yoakim, M., Schaffhausen, B., White, M.F., 1992. IRS-1 activates phosphatidylinositol 3′-kinase by associating with src homology 2 domains of p85. Proc. Natl Acad. Sci. USA 89, 10350–10354.
Myers, M.G., Cowley, M.A., Munzberg, H., 2008. Mechanisms of leptin action and leptin resistance. Annu. Rev. Physiol. 70, 537–556.
Nakanishi, S., Inoue, A., Kita, T., Nakamura, M., Chang, A.C., Cohen, S.N., Numa, S., 1979. Nucleotide sequence of cloned cDNA for bovine corticotropin-beta-lipotropin precursor. Nature 278, 423–427.
Nijenhuis, W.A., Oosterom, J., Adan, R.A., 2001. AgRP(83–132) acts as an inverse agonist on the human-melanocortin-4 receptor. Mol. Endocrinol. 15, 164–171.
Niswender, K.D., Morton, G.J., Stearns, W.H., Rhodes, C.J., Myers Jr., M.G., Schwartz, M.W., 2001. Intracellular signalling. Key enzyme in leptin-induced anorexia. Nature 413, 794–795.
Niswender, K.D., Morrison, C.D., Clegg, D.J., Olson, R., Baskin, D.G., Myers Jr., M.G., Seeley, R.J., Schwartz, M.W., 2003. Insulin activation of phosphatidylinositol 3-kinase in the hypothalamic arcuate nucleus: a key mediator of insulin-induced anorexia. Diabetes 52, 227–231.
Nonogaki, K., Strack, A.M., Dallman, M.F., Tecott, L.H., 1998. Leptin-independent hyperphagia and type 2 diabetes in mice with a mutated serotonin 5-HT2C receptor gene. Nat. Med. 4, 1152–1156.
Opie, E.L., 1900–1901aa. On the relation of chronic interstitial pancreatitis to the islands of langerhans and to diabetes mellitus. J Exper Med 5, 397–428.
Opie, E.L., 1900–1901bb. The relation of diabetes mellitus to lesions of the pancreas; hyaline degeneration of the islets of Langerhans. J Exper Med 5, 527–540.
Orci, L., Baetens, D., Rufener, C., Amherdt, M., Ravazzola, M., Studer, P., Malaisse-Lagae, F., Unger, R.H., 1976. Hypertrophy and hyperplasia of somatostatin-containing D-cells in diabetes. Proc. Natl Acad. Sci. USA 73, 1338–1342.
Overholser, M., 1925. The number of islets of Langerhans in the pancreas of the albino rat. Endocrinology 9, 493–504.
Ozes, O.N., Akca, H., Mayo, L.D., Gustin, J.A., Maehama, T., Dixon, J.E., Donner, D.B., 2001. A phosphatidylinositol 3-kinase/Akt/mTOR pathway mediates and PTEN antagonizes tumor necrosis factor inhibition of insulin signaling through insulin receptor substrate-1. Proc. Natl Acad. Sci. USA 98, 4640–4645.
Plum, L., Ma, X., Hampel, B., Balthasar, N., Coppari, R., Munzberg, H., Shanabrough, M., Burdakov, D., Rother, E., Janoschek, R., Alber, J., Belgardt, B.F., Koch, L., Seibler, J., Schwenk, F., Fekete, C., Suzuki, A., Mak, T.W., Krone, W., Horvath, T.L., Ashcroft, F.M., Bruning, J.C., 2006. Enhanced PIP3 signaling in POMC neurons causes KATP channel activation and leads to diet-sensitive obesity. J. Clin. Investig. 116, 1886–1901.
Qiu, J., Fang, Y., Ronnekleiv, O.K., Kelly, M.J., 2010. Leptin excites proopiomelanocortin neurons via activation of TRPC channels. J. Neurosci. 30, 1560–1565.
Robbins, L.S., Nadeau, J.H., Johnson, K.R., Kelly, M.A., Roselli-Rehfuss, L., Baack, E., Mountjoy, K.G., Cone, R.D., 1993. Pigmentation phenotypes of variant extension locus alleles result from point mutations that alter MSH receptor function. Cell 72, 827–834.
Rossi, M., Kim, M.S., Morgan, D.G., Small, C.J., Edwards, C.M., Sunter, D., Abusnana, S., Goldstone, A.P., Russell, S.H., Stanley, S.A., Smith, D.M., Yagaloff, K., Ghatei, M.A., Bloom, S.R., 1998. A C-terminal fragment of Agouti-related protein increases feeding and antagonizes the effect of alpha-melanocyte stimulating hormone in vivo. Endocrinology 139, 4428–4431.
Rowland, N.E., Carlton, J., 1986. Neurobiology of an anorectic drug: fenfluramine. Prog. Neurobiol. 27, 13–62.
Sasaki, A., Yasukawa, H., Suzuki, A., Kamizono, S., Syoda, T., Kinjyo, I., Sasaki, M., Johnston, J.A., Yoshimura, A., 1999. Cytokine-inducible SH2 protein-3 (CIS3/SOCS3) inhibits Janus tyrosine kinase by binding through the N-terminal kinase inhibitory region as well as SH2 domain. Genes Cells 4, 339–351.
Sasaki, A., Yasukawa, H., Shouda, T., Kitamura, T., Dikic, I., Yoshimura, A., 2000. CIS3/SOCS-3 suppresses erythropoietin (EPO) signaling by binding the EPO receptor and JAK2. J. Biol. Chem. 275, 29338–29347.
Schwartz, G.J., Moran, T.H., 2002. Leptin and neuropeptide y have opposing modulatory effects on nucleus of the solitary tract neurophysiological responses to gastric loads: implications for the control of food intake. Endocrinology 143, 3779–3784.
Schwartz, M.W., Porte Jr., D., 2005. Diabetes, obesity, and the brain. Sci. NY N. Y 307, 375–379.
Schwartz, M.W., Baskin, D.G., Bukowski, T.R., Kuijper, J.L., Foster, D., Lasser, G., Prunkard, D.E., Porte Jr., D., Woods, S.C., Seeley, R.J., Weigle, D.S., 1996a. Specificity of leptin action on elevated blood glucose levels and hypothalamic neuropeptide Y gene expression in ob/ob mice. Diabetes 45, 531–535.
Schwartz, M.W., Seeley, R.J., Campfield, L.A., Burn, P., Baskin, D.G., 1996b. Identification of targets of leptin action in rat hypothalamus. J. Clin. Investig. 98, 1101–1106.
Schwartz, M.W., Seeley, R.J., Woods, S.C., Weigle, D.S., Campfield, L.A., Burn, P., Baskin, D.G., 1997. Leptin increases hypothalamic pro-opiomelanocortin mRNA expression in the rostral arcuate nucleus. Diabetes 46, 2119–2123.
Shaw, J.E., Sicree, R.A., Zimmet, P.Z., 2010. Global estimates of the prevalence of diabetes for 2010 and 2030. Diab. Res. Clin. Pract. 87, 4–14.
Shi, H., Strader, A.D., Sorrell, J.E., Chambers, J.B., Woods, S.C., Seeley, R.J., 2008. Sexually different actions of leptin in proopiomelanocortin neurons to regulate glucose homeostasis. Am. J. Physiol. Endocrinol. Metab. 294, E630–E639.
Skibicka, K.P., Grill, H.J., 2009a. Hindbrain leptin stimulation induces anorexia and hyperthermia mediated by hindbrain melanocortin receptors. Endocrinology 150, 1705–1711.
Skibicka, K.P., Grill, H.J., 2009b. Hypothalamic and hindbrain melanocortin receptors contribute to the feeding, thermogenic, and cardiovascular action of melanocortins. Endocrinology 150, 5351–5361.
Spanswick, D., Smith, M.A., Groppi, V.E., Logan, S.D., Ashford, M.L., 1997. Leptin inhibits hypothalamic neurons by activation of ATP-sensitive potassium channels. Nature 390, 521–525.
Spanswick, D., Smith, M.A., Mirshamsi, S., Routh, V.H., Ashford, M.L., 2000. Insulin activates ATP-sensitive K+ channels in hypothalamic neurons of lean, but not obese rats. Nat. Neurosci. 3, 757–758.
Stanley, B.G., Leibowitz, S.F., 1985. Neuropeptide Y injected in the paraventricular hypothalamus: a powerful stimulant of feeding behavior. Proc. Natl Acad. Sci. USA 82, 3940–3943.
Stephens, T.W., Basinski, M., Bristow, P.K., Bue-Valleskey, J.M., Burgett, S.G., Craft, L., Hale, J., Hoffmann, J., Hsiung, H.M., Kriauciunas, A., et al., 1995. The role of neuropeptide Y in the antiobesity action of the obese gene product. Nature 377, 530–532.
Sutton, G.M., Trevaskis, J.L., Hulver, M.W., McMillan, R.P., Markward, N.J., Babin, M.J., Meyer, E.A., Butler, A.A., 2006. Diet-genotype interactions in the development of the obese, insulin-resistant phenotype of C57BL/6J mice lacking melanocortin-3 or -4 receptors. Endocrinology 147, 2183–2196.
Swanson, L.W., Kuypers, H.G., 1980. A direct projection from the ventromedial nucleus and retrochiasmatic area of the hypothalamus to the medulla and spinal cord of the rat. Neurosci. Lett. 17, 307–312.
Takaya, K., Ogawa, Y., Isse, N., Okazaki, T., Satoh, N., Masuzaki, H., Mori, K., Tamura, N., Hosoda, K., Nakao, K., 1996. Molecular cloning of rat leptin receptor isoform complementary DNAs — identification of a missense mutation in Zucker fatty (fa/fa) rats. Biochem. Biophys. Res. Commun. 225, 75–83.
Tartaglia, L.A., Dembski, M., Weng, X., Deng, N., Culpepper, J., Devos, R., Richards, G.J., Campfield, L.A., Clark, F.T., Deeds, J., Muir, C., Sanker, S., Moriarty, A., Moore, K.J., Smutko, J.S., Mays, G.G., Wool, E.A., Monroe, C.A., Tepper, R.I., 1995. Identification and expression cloning of a leptin receptor, OB-R. Cell 83, 1263–1271.
Tecott, L.H., Sun, L.M., Akana, S.F., Strack, A.M., Lowenstein, D.H., Dallman, M.F., Julius, D., 1995. Eating disorder and epilepsy in mice lacking 5-HT2c serotonin receptors. Nature 374, 542–546.
Thornton, J.E., Cheung, C.C., Clifton, D.K., Steiner, R.A., 1997. Regulation of hypothalamic proopiomelanocortin mRNA by leptin in ob/ob mice. Endocrinology 138, 5063–5066.
Tota, M.R., Smith, T.S., Mao, C., MacNeil, T., Mosley, R.T., Van der Ploeg, L.H., Fong, T.M., 1999. Molecular interaction of Agouti protein and Agouti-related protein with human melanocortin receptors. Biochemistry 38, 897–904.
Tremblay, F., Brule, S., Hee Um, S., Li, Y., Masuda, K., Roden, M., Sun, X.J., Krebs, M., Polakiewicz, R.D., Thomas, G., Marette, A., 2007. Identification of IRS-1 Ser-1101 as a target of S6K1 in nutrient- and obesity-induced insulin resistance. Proc. Natl Acad. Sci. USA 104, 14056–14061.
Tsigos, C., Arai, K., Hung, W., Chrousos, G.P., 1993. Hereditary isolated glucocorticoid deficiency is associated with abnormalities of the adrenocorticotropin receptor gene. J. Clin. Investig. 92, 2458–2461.
Tsujii, S., Bray, G.A., 1989. Acetylation alters the feeding response to MSH and beta-endorphin. Brain Res. Bull. 23, 165–169.
Uhler, M., Herbert, E., 1983. Complete amino acid sequence of mouse pro-opiomelanocortin derived from the nucleotide sequence of pro-opiomelanocortin cDNA. J. Biol. Chem. 258, 257–261.
Ullrich, A., Bell, J.R., Chen, E.Y., Herrera, R., Petruzzelli, L.M., Dull, T.J., Gray, A., Coussens, L., Liao, Y.C., Tsubokawa, M., et al., 1985. Human insulin receptor and its relationship to the tyrosine kinase family of oncogenes. Nature 313, 756–761.
Vaisse, C., Clement, K., Guy-Grand, B., Froguel, P., 1998. A frameshift mutation in human MC4R is associated with a dominant form of obesity. Nat. Genet. 20, 113–114.

van de Wall, E., Leshan, R., Xu, A.W., Balthasar, N., Coppari, R., Liu, S.M., Jo, Y.H., MacKenzie, R.G., Allison, D.B., Dun, N.J., Elmquist, J., Lowell, B.B., Barsh, G.S., de Luca, C., Myers Jr., M.G., Schwartz, G.J., Chua Jr., S.C., 2008. Collective and individual functions of leptin receptor modulated neurons controlling metabolism and ingestion. Endocrinology 149, 1773–1785.

van den Top, M., Lee, K., Whyment, A.D., Blanks, A.M., Spanswick, D., 2004. Orexigen-sensitive NPY/AgRP pacemaker neurons in the hypothalamic arcuate nucleus. Nat. Neurosci. 7, 493–494.

Vickers, S.P., Clifton, P.G., Dourish, C.T., Tecott, L.H., 1999. Reduced satiating effect of d-fenfluramine in serotonin 5-HT(2C) receptor mutant mice. Psychopharmacol. Berl 143, 309–314.

von Mering, J.V., Minkowski, O., 1889. Arch. Exp. Pathol. Pharmakol. 27, 371.

Wade, J.M., Juneja, P., MacKay, A.W., Graham, J., Havel, P.J., Tecott, L.H., Goulding, E.H., 2008. Synergistic impairment of glucose homeostasis in ob/ob mice lacking functional serotonin 2C receptors. Endocrinology 149, 955–961.

Wanting Xu, A., Kaelin, C.B., Takeda, K., Akira, S., Schwartz, M.W., Barsh, G.S., 2005. PI3K integrates the action of insulin and leptin on hypothalamic neurons. J. Clin. Investig. 115, 951–958.

White, M.F., Haring, H.U., Kasuga, M., Kahn, C.R., 1984. Kinetic properties and sites of autophosphorylation of the partially purified insulin receptor from hepatoma cells. J. Biol. Chem. 259, 255–264.

White, M.F., Shoelson, S.E., Keutmann, H., Kahn, C.R., 1988. A cascade of tyrosine autophosphorylation in the beta-subunit activates the phosphotransferase of the insulin receptor. J. Biol. Chem. 263, 2969–2980.

White, D.W., Kuropatwinski, K.K., Devos, R., Baumann, H., Tartaglia, L.A., 1997. Leptin receptor (OB-R) signaling. Cytoplasmic domain mutational analysis and evidence for receptor homo-oligomerization. J. Biol. Chem. 272, 4065–4071.

Wild, S., Roglic, G., Green, A., Sicree, R., King, H., 2004. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diab. Care 27, 1047–1053.

Williams, K.W., Smith, B.N., 2006. Rapid inhibition of neural excitability in the nucleus tractus solitarii by leptin: implications for ingestive behaviour. J. Physiol. 573, 395–412.

Williams, K.W., Zsombok, A., Smith, B.N., 2007. Rapid inhibition of neurons in the dorsal motor nucleus of the vagus by leptin. Endocrinology 148, 1868–1881.

Williams, K.W., Scott, M.M., Elmquist, J.K., 2009. From observation to experimentation: leptin action in the mediobasal hypothalamus. Am. J. Clin. Nutr. 89, 985S–990S.

Williams, K.W., Margatho, L.O., Lee, C.E., Choi, M., Lee, S., Scott, M.M., Elias, C.F., Elmquist, J.K., 2010. Segregation of acute leptin and insulin effects in distinct populations of arcuate proopiomelanocortin neurons. J. Neurosci. 30, 2472–2479.

Woods, S.C., Lotter, E.C., McKay, L.D., Porte Jr., D., 1979. Chronic intracerebroventricular infusion of insulin reduces food intake and body weight of baboons. Nature 282, 503–505.

Woods, S.C., Seeley, R.J., Porte Jr., D., Schwartz, M.W., 1998. Signals that regulate food intake and energy homeostasis. Sci. NY N. Y 280, 1378–1383.

Xu, A.W., Ste-Marie, L., Kaelin, C.B., Barsh, G.S., 2007. Inactivation of signal transducer and activator of transcription 3 in proopiomelanocortin (Pomc) neurons causes decreased pomc expression, mild obesity, and defects in compensatory refeeding. Endocrinology 148, 72–80.

Xu, Y., Jones, J.E., Kohno, D., Williams, K.W., Lee, C.E., Choi, M.J., Anderson, J.G., Heisler, L.K., Zigman, J.M., Lowell, B.B., Elmquist, J.K., 2008. 5-HT2CRs expressed by pro-opiomelanocortin neurons regulate energy homeostasis. Neuron 60, 582–589.

Yeo, G.S., Farooqi, I.S., Aminian, S., Halsall, D.J., Stanhope, R.G., O'Rahilly, S., 1998. A frameshift mutation in MC4R associated with dominantly inherited human obesity. Nat. Genet. 20, 111–112.

Yu, X., Park, B.H., Wang, M.Y., Wang, Z.V., Unger, R.H., 2008. Making insulin-deficient type 1 diabetic rodents thrive without insulin. Proc. Natl Acad. Sci. USA 105, 14070–14075.

Zabolotny, J.M., Bence-Hanulec, K.K., Stricker-Krongrad, A., Haj, F., Wang, Y., Minokoshi, Y., Kim, Y.B., Elmquist, J.K., Tartaglia, L.A., Kahn, B.B., Neel, B.G., 2002. PTP1B regulates leptin signal transduction in vivo. Dev. Cell 2, 489–495.

Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L., Friedman, J.M., 1994. Positional cloning of the mouse obese gene and its human homologue. Nature 372, 425–432.

Zheng, H., Patterson, L.M., Phifer, C.B., Berthoud, H.R., 2005. Brain stem melanocortinergic modulation of meal size and identification of hypothalamic POMC projections. Am. J. Physiol. Regul. Integr. Comp. Physiol. 289, R247–R258.

Zheng, H., Patterson, L.M., Rhodes, C.J., Louis, G.W., Skibicka, K.P., Grill, H.J., Myers, M.G., Jr., Berthoud, H.R., in press. A potential role for hypothalamo-medullary POMC projections in leptin-induced suppression of food intake. Am J Physiol Regul Integr Comp Physiol.

Zhou, L., Williams, T., Lachey, J.L., Kishi, T., Cowley, M.A., Heisler, L.K., 2005. Serotonergic pathways converge upon central melanocortin systems to regulate energy balance. Peptides 26, 1728–1732.

Zhou, L., Sutton, G.M., Rochford, J.J., Semple, R.K., Lam, D.D., Oksanen, L.J., Thornton-Jones, Z.D., Clifton, P.G., Yueh, C.Y., Evans, M.L., McCrimmon, R.J., Elmquist, J.K., Butler, A.A., Heisler, L.K., 2007. Serotonin 2C receptor agonists improve type 2 diabetes via melanocortin-4 receptor signaling pathways. Cell Metab. 6, 398–405.

Zimmet, P., Alberti, K.G., Shaw, J., 2001. Global and societal implications of the diabetes epidemic. Nature 414, 782–787.