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van Dijk, G

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YOUNG INVESTIGATOR PERSPECTIVES

The Role of Leptin in the Regulation of Energy Balance and Adiposity

Gertjan van Dijk
Department of Animal Physiology, Division Neuroendocrinology, School of Behavioural and Cognitive Neurosciences, University of Groningen, Haren, The Netherlands.

Key words: food intake, body weight, hypothalamus, neuropeptide, metabolism.

Abstract

Since its discovery, leptin (a 167-amino acid product of the OB gene) has quickly moved to the forefront as an important hormone for regulation of energy balance. It closes a feedback loop from adipose tissue to hypothalamic neuropeptide-containing neural circuitry involved in regulation of food intake and neuroendocrine/autonomic outflow. While increased central leptin signalling reduces adiposity via a reduction in food intake, it also has remarkable metabolic effects that promote leanness, independent of food intake. These include: (i) increased energy expenditure, (ii) in-place degradation of fat, and (iii) increased thermogenesis. Hypothalamic neurones that synthesize corticotropin releasing hormone and melanocortins (i.e. α-melanocyte-stimulating hormone and agouti-related protein) are likely effector pathways that mediate the anorexigenic and metabolic effects of leptin. Activation of sympathetic outflow (via neuropeptidergic effector pathways of central leptin) to a number of tissues that store fat might be an important mechanism through which these peripheral metabolic effects are elicited. It is proposed that these peripheral metabolic effects contribute to the satiating properties of leptin.

Introduction

In adulthood, higher animals including rodents and primates have a substantial amount of fat in their bodies (between 10% and 15%), mostly stored in white adipose tissue (WAT). An important function of fat is to buffer digested nutrients in the form of triglycerides. After hydrolysis of triglycerides into free fatty acids (FFA) and glycerol, FFA can be used as fuel for oxidative phosphorylation, which provides the basic source of energy (i.e. ATP). The amount of energy stored in the form of triglycerides and potentially employable for metabolic purposes is enormous compared to that of glycogen, another important fuel depot in the body. If no such fat store were available, intake from the environment would have to be continuous. This is obviously not the case because food intake occurs mostly in specific bouts (i.e. meals) that are interspaced by relatively long time intervals. Thus, controlled hydrolysis and oxidative phosphorylation allows continuous energy expenditure (i.e. during and between meals and even under conditions of prolonged starvation) needed for muscular movement, reproduction, growth, cellular maintenance and for keeping body temperature in the optimal physiological range.

An intriguing fact about fat is that many individuals have a constant amount of it in their bodies over most of their lifespan. One implication of this is that the amount of triglycerides utilized for metabolic purposes is exactly met by the synthesis of triglycerides from fuels directly or indirectly derived from ingested nutrients. Over the past few years, evidence has accumulated that the obesity (OB) gene, a highly conserved DNA region in many species (in humans and mice found on chromosome 6 and 7, respectively) plays a crucial role in the match between triglyceride depletion and storage. The OB gene encodes a highly conserved 4.5 kb adipose tissue messenger which is translated into the 167-amino acid product, leptin. Whereas leptin is able to regulate triglyceride storage via a paracrine action (1, 2), the present review focuses

Correspondence to: Gertjan van Dijk, Department of Animal Physiology, PO Box 14, 9750 AA Haren, The Netherlands (e-mail: g.van.dijk@biol.rug.nl).

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on the interaction of leptin with the central nervous system (CNS). This feedback of leptin on CNS circuitry is suggested to control energy balance and body adiposity via adaptations in both food intake and food intake-independent mechanisms in a coordinated fashion.

The concept of leptin action in the CNS

Several decades ago, Kennedy recognized the stability of body adiposity and energy balance (3). At that time, it was already known from the pioneering work of Hetherington and Ranson that electrolytic lesioning of the ventrobasal hypothalamus including the arcuate (Arc), dorsomedial (DMH) and ventromedial (VMH) nuclei causes rats to become dramatically hyperphagic and obese (4). Based on these data, Kennedy proposed the idea that there is a factor proportional to the size of body fat stores that signals to the hypothalamus (3). Changes in this fat signal would lead to appropriate changes in food intake to maintain body adiposity level. Evidence that regulation of adiposity involves a hormonal feedback signal in the body has been obtained from experiments with parabiotic rats. These are surgically united pairs of rats which permanently exchange blood through a capillary anastomosis. If one rat is made obese by a lesion in the ventrobasal hypothalamus, the other becomes very thin, apparently through reducing its food intake and increasing energy expenditure in response to the obesity in the partner, with which it shares a blood supply (5). Several candidate lipostat factors (e.g. insulin, steroids) have been hypothesized throughout the years, but it was the discovery of leptin by Zhang *et al*. in 1994 (6) that significantly reinforced Kennedy's concept.

Leptin mRNA levels in adipocytes and the concentration of plasma leptin change rapidly over the diurnal cycle and may reflect rapid changes in energy balance associated with fasting and refeeding (7). Accordingly, a fall in plasma leptin has been suggested to signal starvation (8). In the longer term, however, the integrated level of plasma leptin reflects body adiposity. While a number of hormones (e.g. insulin, corticosterone) are involved in the synthesis of leptin, the principal determinant of leptin production has yet to be discovered. Among the tissues that express mRNA encoding the signalling form of the leptin receptor (OB-Rb; among five splice variants, the only form known to activate transcription factors) are several peripheral tissues and organs (e.g. adipose tissue, liver, muscle) and a number of regions in the CNS, including the ventrobasal (i.e. Arc, VMH, DMH) hypothalamus (8). The role of leptin and its receptors in regulation of energy balance is well-illustrated by observations that rodents that have mutations in either their signalling leptin receptors [i.e. *db*/*db* mice or *fa*/*fa* Zucker rats (9)] or in leptin production [i.e. *ob*/*ob* mice (10)] are extremely obese. Apparently, these animals lack a negative feedback signal from their adipose tissue stores to the CNS, which would be necessary to allow appropriate adjustments to disturbances (i.e. obesity) in adipose tissue mass. Recombinant functional leptin administration obviously only reverses obesity in animals that synthesize a deficient form of leptin, but not in animals with a deficient leptin receptor (9, 10). Although extremely rare, mutations in leptin synthesis leading to morbid obesity have also been reported in humans; for a review, see (11). The role of leptin in regulation of adiposity of animals without defects in leptin synthesis or signalling has primarily been investigated in rodents. As an example, Koyama *et al*. (12) showed that normal lean rats lose virtually all fat when these animals undergo adenovirus gene transfer of the OB gene. This treatment, however, is not effective in animals with an electrolytic lesion in the ventrobasal hypothalamus, demonstrating the importance of this brain region in mediating the effects of leptin on energy balance (12).

An effective way to reduce body fat is through a reduction in food intake, and there are many studies that consistently demonstrate the anorexigenic efficacy of leptin (8, 11, 13). Of importance for considering the specificity of leptin as a putative anorexigenic hormone is our observation that leptin effects on food intake are not due to incapacitation or induction of malaise (14). Furthermore, relatively low doses of leptin delivered into the third cerebral ventricle (i3vt) of rats (9) and mice (10), and even lower doses in the VMH and Arc (15), are much more potent to reduce food intake than when leptin is delivered peripherally. This indicates that the VMH/Arc region is the target where leptin acts. Other evidence pointing in this direction are findings that i3vt (16) and intravenous (13) administration of anorexigenic doses of leptin increases labelling of c-Fos (a marker for neuronal activity) in several hypothalamic regions including the ventrobasal hypothalamus.

Effects of leptin in the CNS on substrate metabolism and fuel handling

In addition to being hyperphagic, leptin-deficient rodents utilize dietary energy more efficiently than wild-type animals (17), which suggests effects of leptin on body adiposity independent of food intake. The findings that leptin treatment of *ob*/*ob* mice is able to increase oxygen consumption in these animals, and induces leanness more potently than is expected based on the reduction in food intake alone (18), may imply that brain leptin has profound metabolic effects. Three aspects of central leptin effects on adiposity that are independent of food intake are described below.

Energy expenditure

In a recent study, we have investigated the central effect of leptin on energy balance and body adiposity in genetically normal rats independent of leptin effects on food intake (19). Briefly, rats were treated i3vt with leptin (3 μg/day) or with synthetic cerebrospinal fluid (sCSF, the vehicle in which leptin was dissolved) over a 3-day period during which animals ate approximately 50% of the amount eaten by sCSF-treated, *ad libitum* fed controls. These anorexigenic effects by itself have major consequences for metabolism and energy expenditure. Therefore, an additional group of animals received the vehicle, but was pair-fed to the i3vt leptin-treated group, and thus served to investigate the effect of leptin on metabolism independent of the effect of leptin on food intake. On the last day of treatment, animals were put into an indirect calorimetry chamber for a 3-h recording of carbohydrate oxidation (CHO-ox), fat oxidation (Fat-ox) and energy expenditure

(EE), during which the animals were not allowed to eat. Meanwhile, behaviour of these animals was videotaped so that the measurement of CHO-ox, Fat-ox and EE could be related to when the animals were under resting conditions. It was observed that i3vt leptin-treated animals, while eating only approximately 50% of the food that was eaten by the *ad libitum* controls, had a level of EE that was indistinguishable from *ad libitum* controls during the resting phase. In contrast, the pair-fed controls had a significantly lower level of EE relative to both other groups during the resting phase. Since there were no differences in time spent on resting, grooming and alertness among treatment groups, these data can be interpreted to indicate that the fall in resting EE that is normally associated with reduced caloric intake is prevented by i3vt leptin treatment. Consistent with these effects was the observation that i3vt leptin-treated animals were leaner than their pair-fed controls at the end of treatment. Body fat analysis and energy expenditure are depicted in Fig. 1.

With respect to the specific utilization of carbohydrate and fat, only the pair-fed group, but not the leptin-treated group, had a significantly lower CHO-ox relative to *ad libitum* controls, whereas the level of Fat-ox was only significantly increased in the leptin-treated, but not in the pair-fed group, relative to the *ad libitum* controls. Thus, these data indicate that the relative high EE in leptin-treated animals versus that of pair-fed animals occurred at the expense of both fat as well as carbohydrate (Fig. 2).

In-place utilization of fat

In addition to the lower resting EE, pair-fed animals had elevated levels of plasma FFA, ketones, and glycerol compared to *ad libitum* controls, and these are considered normal responses to food restriction where triglyceride breakdown, ketone body production and β-oxidation predominate. In contrast, the concentration of plasma FFA and ketones of i3vt leptin-treated animals was at the level of *ad libitum* controls. Because the plasma level of glycerol was elevated to a similar extent in leptin-treated and pair-fed animals, leptin apparently did not prevent the intracellular breakdown of triglycerides (Fig. 3). In fact, lipid depletion and Fat-ox appeared to be accentuated by central leptin treatment

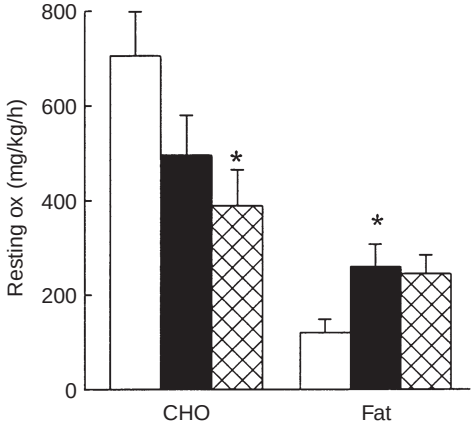

FIG. 2. Carbohydrate oxidation (CHO-ox) and Fat oxidation (Fat-ox) of *ad libitum* feeding male Wistar rats that received daily intracerebroventricular infusion of synthetic cerebrospinal fluid (sCSF, open bars; n = 8) or 3.5 μg leptin (filled bars; n = 8). An additional group of rats received intracerebroventricular infusion of sCSF and were pair-fed to leptin-treated animals (shaded bars; n = 8). CHO-ox and Fat-ox were determined using indirect calorimetry over a 3-h period on the last day of treatment. Statistical significance between *ad libitum*/sCSF-treated and other groups; *P < 0.05. Adapted from (19) with permission.

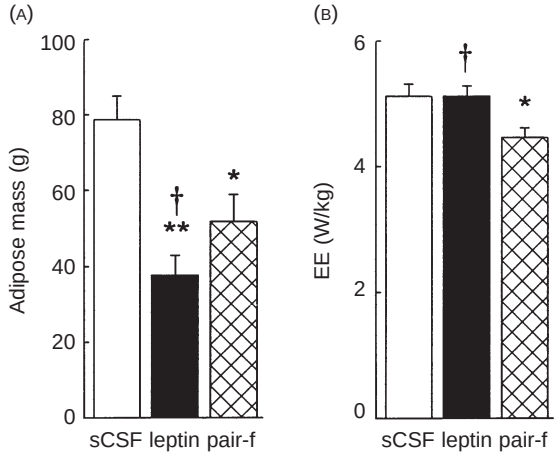

FIG. 1. The percentage of body adiposity and resting energy expenditure of *ad libitum* feeding male Wistar rats that received daily intracerebroventricular infusion of synthetic cerebrospinal fluid (sCSF, open bars; n = 8) or 3.5 μg leptin (filled bars; n = 8). An additional group of rats received intracerebroventricular infusion of sCSF and were pair-fed to leptin-treated animals (shaded bars; n = 8). Statistical significance between *ad libitum*/sCSF-treated and other groups; *P < 0.05. Statistical significance between leptin and pair-fed group: †P < 0.05. Adapted from (19) with permission.

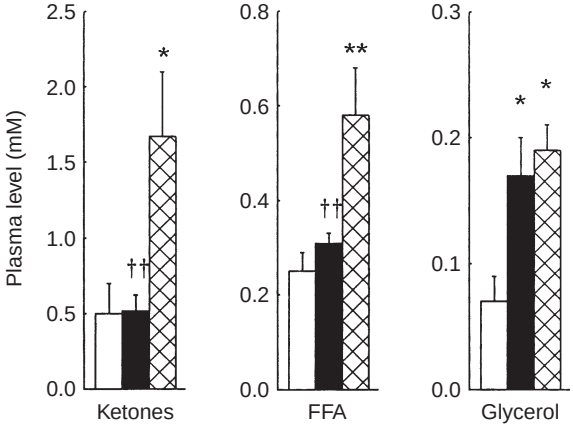

FIG. 3. Plasma concentrations of ketones, free fatty acids (FFA), and glycerol of *ad libitum*-feeding male Wistar rats that received daily intracerebroventricular infusion of synthetic cerebrospinal fluid (sCSF, open bars; n = 8) or 3.5 μg leptin (filled bars; n = 8). An additional group of rats received intracerebroventricular infusion of sCSF and were pair-fed to leptin treated animals (shaded bars; n = 8). Statistical significance between *ad libitum*/sCSF-treated and other groups; *P < 0.05 and **P < 0.01. Statistical significance between leptin and pair-fed group: ††P < 0.01. Adapted from (19) with permission.

as judged from the analysis of body fat and indirect calorimetry depicted in Figs 1 and 2, respectively. One possible interpretation pertinent to the outcome of these results might be that central leptin stimulates lipolysis and concomitantly increases FFA uptake from the blood stream into metabolically active tissue which, in turn, leads to a reduction in plasma FFA levels. However, increased uptake of FFA into the liver would most certainly lead to a rise in plasma level of ketone bodies in leptin-treated animals, and that is not what happened. The second interpretation might be that central leptin stimulates lipolysis while at the same time reducing FFA release from adipocytes. In turn, increased intracellular FFA availability inside adipocytes as well as other tissues that store fat (e.g. muscle, liver, pancreas) could serve as a fuel for increased β-oxidation (i.e. in-place utilization) within the same cells. Thus, rather than increasing the export of FFA to other tissues, these data suggest that leptin acts on CNS pathways that stimulate intracellular triglyceride metabolism in white adipose tissue (and perhaps other tissues that store fat). The reduction in plasma ketone levels by central leptin treatment was probably secondary to the reduced availability of FFA to the liver where increased FFA availability would normally result in increased ketogenesis.

The effects of leptin administration on circulating fat fuels mentioned above confirm and extend the findings of Shimabukuro *et al*. (2), who, using rats made hyperleptinemic by adenovirus gene transfer, found evidence for a mechanism in which leptin increases intracellular fat oxidation in tissues (i.e. in-place utilization) such that lipolysis proceeds without increased levels of plasma FFA and ketones. Whereas it has been proposed that this occurs via a direct action of leptin in peripheral tissues (1, 2), the results from our studies demonstrate that these metabolic responses can be mediated via an action in the CNS. One mechanism to facilitate breakdown of triglycerides is through stimulation of the sympathetic nervous system (SNS) (20). Although the significance of sympathetic innervation of WAT has been questioned for many years, recent work by Bartness *et al*. has led to true neuroanatomical evidence for the origins of sympathetic nervous system outflow from the CNS to WAT (21). They injected a retrograde transsynaptic tracer (pseudorabies virus) in WAT of Siberian hamsters and observed labelling in a number of CNS regions including the intermediolateral cell column of the spinal cord and in the paraventricular nucleus of the hypothalamus (PVN). Since leptin administration into the CNS stimulates regional sympathetic nerve traffic to a number of organs (22) and causes an increase in the circulating level of norepinephrine (23), it might be hypothesized that leptin stimulates SNS outflow to WAT. This idea is consistent with the finding of Commins *et al*. who found that leptin administration lowered its own expression in WAT, provided the sympathetic nervous system remained intact (24).

Thermogenesis

In addition to being severely obese, rodents with a deficient leptin system (e.g. *ob*/*ob* mice) have lower body temperatures in conditions of room (i.e. 20–22°C) temperature and are not capable of maintaining their body temperature when subjected to a cold environment (25). These observations suggest that leptin is involved in production of body heat. A well-known mechanism to increase thermogenesis is to render metabolism less efficient such that part of the energy derived from food is released as heat rather than in the form of ATP. In 1985, Klingenberg and Winkler discovered that the mitochondria of brown adipose tissue (BAT) are metabolically inefficient because of the presence of uncoupling protein (UCP) in the inner mitochondrial wall (26). Activation of UCP disrupts the electrochemical gradient (necessary to drive ATP production) due to the fact that UCP transports protons across, thereby transmitting heat. Subsequently, it was found that UCP activity in BAT adipocytes is stimulated by cold, increased SNS tone, and by leptin (27). Collins *et al*. have shown that leptin signalling in the CNS is able to increase BAT UCP synthesis, and SNS innervation of BAT is required for this effect (28).

The contribution of BAT UCP in the generation of total body heat has long been debated since BAT is present in only very small amounts in adult animals (27, 29). Thus, the failing interaction of leptin with BAT UCP would hardly, if at all, provide an explanation for the hypothermia observed in leptin-deficient animals. In recent years, however, other subtypes of UCP have been cloned and found in almost any other tissue involved in regulation of energy balance. In particular UCP2 and UCP3 are of interest since, besides being present in BAT, these subtypes are found in WAT, muscle and in the liver (27). The work of Cusin *et al*. provided a functional link between central leptin action and whole body UCP activity in rats (30). These researchers demonstrated that CNS leptin administration (12 μg/day into the lateral ventricle during a 4-day period) was able to stimulate BAT UCP (referred to as UCP1), UCP2 and UCP3 in BAT, WAT, muscle and liver, respectively, relative to animals that were pair-fed to the leptin-treated group (30). In our hands, this treatment significantly increased body temperature independent of locomotor activity, which is consistent with leptin stimulation of UCP activity (Fig. 4). Analogous to the contribution of the SNS to leptin-mediated stimulation of UCP1 in BAT, the same mechanism might be expected to occur in WAT, muscle and the liver. Data are emerging showing that thyroid hormone secretion might be an additional mechanism through which leptin affects UCP activity (31).

CNS targets of leptin involved in the regulation of adiposity

Several studies indicate that leptin mediates its effect on food intake through modulatory actions on the synthesis of a number of neuropeptides produced by neuronal cell bodies in the base of the hypothalamus (including the Arc). One leptin-binding neuronal cell type synthesizes neuropeptide Y (NPY) and agouti-related protein (AgRP). NPY and AgRP are potent stimulants of food intake and promote body weight gain when administered into the CNS, while reduced leptin signalling (e.g. in fasting or leptin-deficient rodents) consistently upregulates NPY and AgRP mRNA in Arc neurones. This upregulation of NPY and AgRP mRNA can be reversed by i3vt administration of leptin. Another leptin-receptive cell type cosynthesizes cocaine-amphetamine related transcript (CART) and pro-opiomelanocortin (POMC), from which

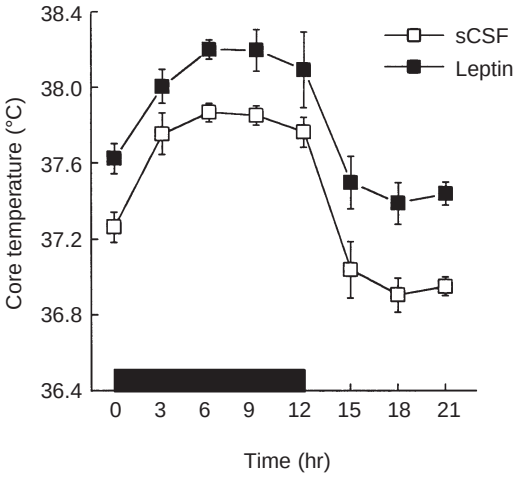

FIG. 4. Twenty-four hour core temperature patterns of *ad libitum* feeding male Wistar rats averaged over three-hour periods (assessed with DATAQUEST biotelemtry system) at the seventh day of chronic third-cerebroventricular infusion (via osmotic minipumps; Alzet, model 2002) with synthetic cerebrospinal fluid (sCSF; n = 6) or leptin (10 μg/day, n = 5). At this particular day of treatment, the animals infused with leptin consumed 52% of the amount eaten by sCSF-treated controls. The black bar on the horizontal axis denotes the dark period. (de Vries *et al*., in preparation).

α-melanocyte stimulating hormone (αMSH) is cleaved. While CART and αMSH reduce food intake and promote loss of adiposity upon i3vt administration, leptin consistently up-regulates the synthesis of these neuropeptides in fasting or leptin-deficient rodents (8, 11, 13). The neuronal cell bodies that produce NPY/AgRP and CART/POMC project intra-hypothalamically as well as extra-hypothalamically (13) and may affect food intake directly through changing the sensation of hunger and/or satiety. Alternatively, they might affect hunger/satiety feelings indirectly by altering the activity of classic neurotransmitter systems (e.g. that contain norepinephrine, dopamine, serotonin and GABA) and/or neuropeptide systems that contain for instance melanin-concentrating hormone (32) and corticotropin releasing hormone (CRH) (33, 34). Many of these hypothalamic neuropeptidergic systems have been included in mediating leptin effects on adiposity independent of their effects on food intake. The most extensively studied ones are NPY, POMC and CRH.

NPY

The first described neuropeptide with potent effects on energy balance independent of food intake is NPY (35). NPY and its receptors are abundantly expressed throughout the CNS. Most relevant to regulation of energy balance are NPY-containing neuronal cell bodies in the Arc that project heavily to the PVN (36). When injected into the PVN of rats, NPY reduces energy expenditure (37) and shifts the autononomic balance more towards parasympathetic activity (38); the latter particularly occurring during food intake (38). Consistent with its metabolic and autonomic effects are findings by Zarzjevski *et al*. (39) demonstrating that, relative to controls, chronic NPY administration into the lateral cerebral ventricle produces hyperinsulinemia and increased lipogenesis in rats in which their hyperphagia is prevented by pair-feeding to controls. Besides affecting fuel partitioning and metabolism, centrally administered NPY reduces sympathetic outflow to BAT, thereby probably limiting its thermogenic capacity (40). The defective thermogenic response in fatty Zucker rats is believed to be caused, in part, by a failure to attenuate NPY transmission at the level of the hypothalamus (41). The latter impairment is probably a direct result of ablation of leptin action in the fatty Zucker rat (42).

Although studies in leptin-deficient or fasting rodents clearly demonstrate that leptin reduces NPY synthesis and transmission (43), it is not clear whether the interaction of leptin with NPY synthesis in the Arc of genetically normal, nonfasting rats is of major importance for the day-to-day regulation of energy balance. For example, while food restriction of genetically normal rats clearly increases NPY mRNA levels in the Arc nucleus of these animals (as can be observed in rats that are pair-fed to match the reduced food intake in i3vt leptin-treated animals [19]), i3vt leptin treatment is not able to reverse the increased level of NPY mRNA to the level of *ad libitum* controls (19). In contrast, i3vt leptin treatment did significantly increase POMC and CRH mRNA in the same animals compared to that of pair-fed controls (19). These data might suggest that the interaction of leptin with hypothalamic POMC and CRH synthesis, rather than with NPY synthesis, is more relevant to the day to day regulation of energy balance in genetically 'normal' rats. Of course, it remains possible that leptin can influence the secretion of NPY from nerve terminals without detectable changes in the synthesis of NPY.

POMC

αMSH is one of several cleavage products of POMC, and may be the most relevant for regulation of energy balance relative to the other products. It acts as an agonist on brain melanocortin (MC) receptors (44), and competes with AgRP, which acts as an endogenous antagonist on MC receptors (45). This system has received much attention over the last few years since obesity can occur from different mutations in the MC pathway in humans (46). In addition, mice with a targeted deletion in the gene encoding for the MC4 receptor become morbidly obese (47). The MC4 receptor is widely expressed in the brain and is found in the cortex, hippocampus, thalamus, hypothalamus, spinal cord and peripheral sympathetic nervous system (13). In the hypothalamus, MC4 receptors are highly expressed in neuronal cell bodies of the DMH, PVN and lateral hypothalamus, which all receive input from arcuate hypothalamic neurones that produce αMSH and AgRP (48). In addition to these intrahypothalamic projections, these neurones also project to regions outside the hypothalamus, such as the intermediolateral cell column of the spinal cord, which contains preganglionic motor neurones of the sympathetic nervous system (13).

There are several lines of evidence demonstrating the involvement of the brain MC system in leptin action. The first observation came from our rat study in which the anorexia

following an acute i3vt injection of leptin was prevented by coadministration of the MC-R3/4 receptor antagonist, SHU9119 (Ac-Nle4-c[Asp-His-D-Nal(2′)-Arg-Trp-Lys-NH_2, 0.5 nmol), in a dose which was ineffective by itself to alter food intake, when given i3vt acutely (49). Satoh *et al*. showed that central SHU9119 treatment was also able to block the effect of leptin of increasing UCP1 synthesis in BAT (50). There is currently no data to show whether SHU9119 is able to block the effect of leptin on UCP2 and UCP3. Nevertheless, Haynes *et al*. found that the central stimulatory effects of leptin on regional sympathetic nerve traffic in some, but not all, tissues tested could at least partially be blocked by SHU9119 pretreatment (51). The latter two observations indicate that MC receptors are directly involved in mediating the metabolic effects of leptin.

In a recently published follow-up experiment, we investigated the effect of chronic i3vt administration of SHU9119 (0.5 nmol/day) during an 11-day period on food intake, body weight and temperature regulation in rats (52). Whereas acute i3vt injection of 0.5 nmol SHU9119 did not affect food intake itself but was able to block the anorexigenic effect of leptin (49), i3vt SHU9119 given chronically (by osmotic minipumps) caused rats to slowly increase their food intake to finally almost double the amount at the end of treatment relative to sCSF-treated/*ad libitum*-fed controls. As a consequence, there was a marked increase in body weight and body fat content and a six-fold increase in the plasma leptin level compared to those of controls (52). These data are consistent with other studies investigating food intake and body weight effects of chronic centrally administered receptor antagonists (53). Furthermore, i3vt SHU9119-treated rats in our study had a reduction in body temperature relative to controls, and this effect was even more pronounced in the SHU9119-treated animals that were pair-fed to controls (Fig. 5).

The effects of i3vt SHU9119 treatment to reduce body temperature were particularly pronounced during the dark (i.e. active) phase; i.e. at the time when also the most dramatic increases in food intake were observed in SHU9119-treated/*ad libitum* feeding animals. The observation that the SHU9119-treated/pair-fed animals had significantly more body fat and a two-fold increase in the concentration of plasma leptin compared to those of sCSF-treated controls is probably the result of reduced thermogenesis, which is an indirect measurement of metabolic rate (52). These data are consistent with a recent study of Ste Marie *et al*., showing that wild-type mice are leaner than MC4 receptor knockout mice that were pair-fed to the wild-type mice (54). Taken together, these data indicate that inhibition of MC receptor activity reduces brain leptin effects on thermogenesis and metabolic rate. The notion that leptin activates MC circuitry has been proven to be correct via electrophysiological recordings of POMC neurones, which were identified by targeted expression of green fluorescent protein in transgenic mice (55).

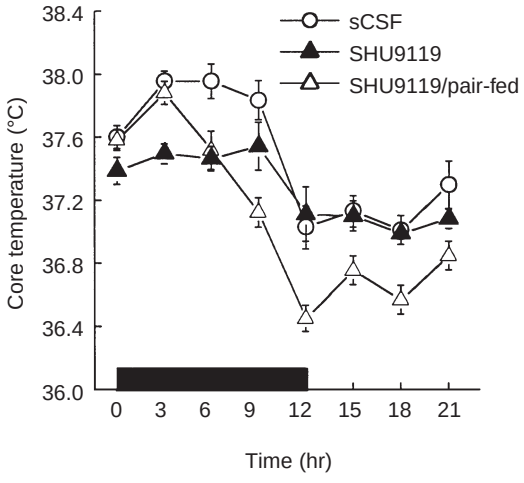

FIG. 5. Twenty-four hour core temperature patterns of *ad libitum* feeding male Wistar rats averaged over three-hour periods (assessed with DATAQUEST biotelemtry system) at the seventh day of chronic third-cerebroventricular infusion (via osmotic minipumps; Alzet, model 2002) with synthetic cerebrospinal fluid (sCSF, n = 6) or SHU9119 (0.5 nmol/day, n = 7). At this day and particularly during the dark phase, SHU9119-treated/*ad libitum* feeding animals ate 72% more than the amount of food consumed by sCSF-treated controls. A third group of animals was infused with SHU9119 but was pair-fed to the sCSF-treated *ad libitum* control group (SHU9119/pair-fed). The black bar on the horizontal axis denotes the dark period. Adapted from (52) with permission.

CRH

CRH is produced in PVN neurones that project to several brain regions, including the median eminence to regulate ACTH secretion, and to the spinal cord to activate sympathetic nervous system outflow (56). Important evidence for involvement of CRH transmission in leptin action on food intake has been provided in two separate studies by Uehara *et al*. (33) and Gardner *et al*. (34). Both studies employed CRH receptor antagonists to block leptin effects on food intake, analogous to our study in which we were able to block leptin effects on food intake with SHU9119 (49). Although PVN neurones have remarkably low expression levels of Ob-Rb mRNA, it has been shown in a number of studies that i3vt leptin administration increases CRH mRNA in the parvocellular PVN neurones (8, 19). This might suggest that other leptin-receptive circuitry is required for activation of CRH-containing PVN neurones. There is indeed evidence that MC receptors, possibly via modulatory effects of interneurones, can affect the activity of PVN neurones (57). Linkage between leptin signalling through MC circuitry and activation of PVN neurones is shown by our finding that i3vt SHU9119 administration is able to blunt the leptin-induced c-Fos labelling of neurones in the PVN (49). At present, reports on direct linkage between leptin signalling through MC receptors and activation of PVN CRH neurones are not available. However, we recently observed that i3vt administration of leptin (3.0 μg) and the synthetic MC-R3/4 receptor agonist, melanotan II (Ac-Nle4-c[Asp^5, D-Phe^7, Lys^{10}]αMSH-(4-10)-NH_2, 0.5 nmol) equated to produce similar reductions in food intake caused similar c-Fos labelling, in approximately 70% of the PVN neurones that colabel CRH (van Dijk *et al*., unpublished data). Thus, the data taken together provide strong evidence

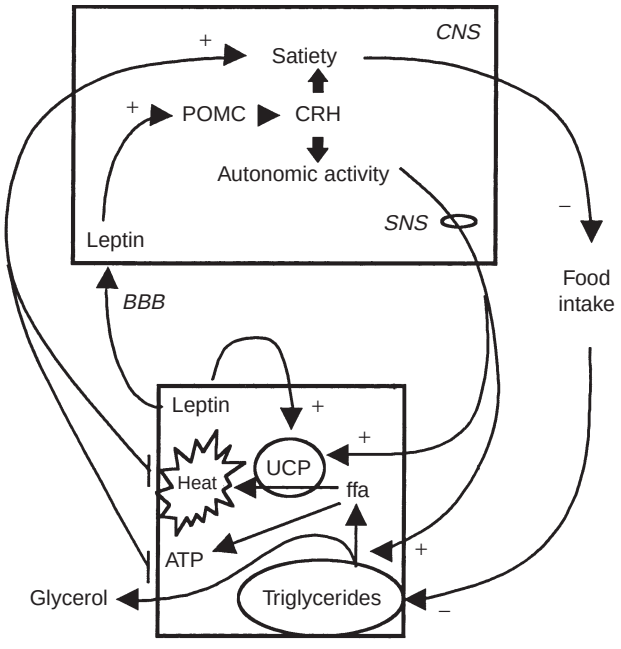

FIG. 6. Summary model of leptin affecting central nervous effector pathways that act directly to inhibit food intake and, presumably via increased sympathetic outflow, alter peripheral substrate metabolism ('in-place oxidation') and produce body heat and ATP. In turn, these peripheral processes elicit feedback signals that, in concert with the direct anorexigenic effect of leptin in the brain, are hypothesized to sustain negative energy balance and depletion of body fat stores. CNS, central nervous system; SNS, sympathetic nervous system; BBB, blood–brain barrier; ffa, free fatty acids; UCP, uncoupling protein, CRH, corticotropin releasing hormone; POMC, pro-opiomelanocortin.

that the MC system serves a pivotal role in linking leptin signalling to activation of CRH neurones.

Since injection of CRH into the cerebral ventricular system stimulates thermogenesis and oxygen consumption and potently reduces food intake and body weight (58, 59), it can be suggested that activation of the melanocortin-CRH loop is an important system to promote body weight loss. In this respect, the trans-synaptic tracer studies by Bamshad *et al.* [which showed some of the strongest labelling in the parvocellular division of the PVN when the pseudorabies tracer was injected in WAT or BAT (21)] are of interest. Although the true identity of these peripherally projecting PVN neurones remains to be investigated, it is very possible that these parvocellular neurones contain CRH.

Conclusions and perspectives

Leptin has a major role in regulating energy balance via modulatory actions on hypothalamic neuropeptide-containing circuitry. In addition to the direct anorexigenic effects elicited by leptin action on this circuitry, the reviewed experiments demonstrate that increased central leptin signalling stimulates food intake-independent mechanisms that promote weight loss. These include an increase in energy expenditure, thermogenesis and (at least in some tissues) in-place degradation of triglyceride stores. In particular, CRH produced in paraventricular hypothalamic neuronal cell bodies might be important in mediating the central anorexigenic and catabolic effects of leptin, the latter presumably via activation of sympathetic outflow to BAT, WAT, liver and muscle. The brain MC system may provide a pivotal link between CNS leptin signalling and activation of CRH neurones. Disturbances in this link (e.g. receptor polymorphisms, receptor downregulation) may be a major contributor to human obesity.

Among the many factors capable of reducing food intake is an increase in the hepatic oxidation of fuels, including fat fuels (60) and an increase in body temperature (61). The fact that increased central leptin signalling increases fuel oxidation and increases body temperature gives the rise to the possibility that these metabolic and thermogenic effects induced by leptin action in the CNS could elicit anorexigenic actions by themselves. According to the theory of thermoregulatory control of food intake (29), it would be predicted that a thermogenic compound would cause rats to eat smaller meals, and this is exactly what i3vt leptin infused rats do (62). Thus, as shown in Fig. 6, the peripheral effects mediated centrally by leptin, in concert with changes in hypothalamic effector pathways that act directly to inhibit food intake, are hypothesized to sustain the negative energy balance and depletion of triglyceride stores characteristic of leptin actions in the brain.

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