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Obesity and the Hypothalamus: Novel Peptides for New Pathways

Minireview

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The belief that the hypothalamus plays a role in the regulation of energy homeostasis was originally based upon the results of brain lesions. Disruption of the ventromedial hypothalamus produced hyperphagic obesity, while lesions of the lateral hypothalamus caused hypophagia and weight loss, suggesting the existence of ventromedial "satiety" and lateral "feeding" centers. Although this framework is now recognized as simplistic, it is the scaffold upon which an understanding of the complex neurochemical system underlying regulation of appetite and energy expenditure has been built. Initially, physiologists examined the responses to injection of neurochemicals present in and around the hypothalamus, using feeding, autonomic output, and hormonal measurements as a final readout. Such approaches defined the effects of the neurotransmitters norepinephrine, dopamine, and serotonin, as well as neuropeptides including neuropeptide Y (NPY), corticotropin-releasing hormone (CRH), and galanin; and elements of a neural wiring diagram began to take shape. Indeed, it appeared possible, until several years ago, that the key molecular mediators in the brain were known, and only their mode of interaction remained to be defined. But the stability of body weight in the face of variable nutritional supplies suggested that additional molecular mediators and regulatory pathways remained undiscovered. This suspicion has been validated by a number of recent discoveries, including one in this issue of *Cell* (Sakurai et al., 1998). Through a combination of genetic and biochemical approaches, novel molecular mediators and pathways have been identified in the periphery and the brain, heralding an exciting new phase of discovery and applied research.

Leptin Is a Peripheral Signal Informing the Brain of Energy Stores

The *ob* and *db* genes encode an adipocyte-derived hormone (e.g., leptin) and its receptor (Zhang et al., 1994; Caro et al., 1996, and references therein). Leptin expression and function are essential for the avoidance of obesity, insulin-resistant diabetes, and infertility, an understanding that has profoundly changed the field of nutritional physiology. Although the consequences of the absence of leptin signaling are clear, determining the normal physiological role for leptin required further experimentation. One model is that circulating leptin levels rise with nascent obesity, engaging receptors in the hypothalamus, where actions are initiated that limit food intake and alter autonomic output (Zhang et al., 1994). The latter stimulates increased heat production (thermogenesis), altered hormone (especially insulin) secretion and action, and partitioning of energy stores away from fat, all of which resist obesity. However, a second important role for leptin may be to signal starvation to the brain (Ahima et al., 1996). Falling leptin evokes a distinct set of adaptive responses, including diminished reproductive capacity and lower thyroid hormone levels, to conserve energy for survival. Both roles for leptin may exist, each at a different end of its biologic dose–response curve. However, because starvation poses a more immediate and frequent threat, the response to starvation might exceed in importance the avoidance of obesity from an evolutionary perspective.

In any case, an understanding of obesity requires that the status of the leptin system in patients with obesity be explained. In the great majority of obese humans and in most rodent models, leptin levels are increased, indicating that most obesity is leptin resistant (Maffei et al., 1995). Two potential mechanisms for this resistance have been explored. The first is a defect in the blood–brain barrier transport system for bringing leptin to CNS sites of action. That such a defect exists is supported by the fact that high levels of serum leptin observed in obesity are not paralleled by proportional rises in CSF leptin (Caro et al., 1996). The molecular basis for a transport defect is unknown. A second site for leptin resistance involves defects in the sites of leptin action within the CNS. As a result, great attention has been focused upon the molecular targets of leptin action in the brain.

NPY: The Initial Target

Initially, among several possible mediators of leptin action within the hypothalamus, NPY was the most compelling (reviewed in Erickson et al., 1996). Several properties suggested that it might be an essential conduit for the leptin signal. Injection of NPY centrally evokes virtually all of the features of leptin deficiency, including hyperphagia, decreased brown adipose tissue (BAT) thermogenesis, and hyperinsulinemic insulin resistance. Repetitive injection causes obesity. Its regulation fits as well, since arcuate nucleus NPY is increased by starvation and in the leptin-deficient *ob*/*ob* mouse, and leptin repletion restores this expression to normal. Thus, it surprised most investigators when NPY knockout mice lacked a feeding or obesity phenotype. Increased NPY does contribute to the *ob*/*ob* phenotype, since *ob*/*ob* mice that also are NPY^{-}/NPY^{-} are substantially less obese and have improvement (but not complete reversal) of the *ob*/*ob* neuroendocrine phenotype (Erickson et al., 1996). However, the obesity in these mice is severe nonetheless, and NPY^{-}/NPY^{-} mice respond normally (or even excessively) to the satiety effects of leptin. Thus, other targets of leptin must exist within the brain for bringing about the responses to both low and high leptin levels.

The agouti, POMC, Melanocortin Connection

In parallel with progress on leptin and NPY, a second front has developed rapidly in the realm of leptin targets in the hypothalamus. The initial observation came from the *lethal yellow* (A^{y}/a) mouse, an autosomal dominant obesity model. Obesity in these mice is accompanied

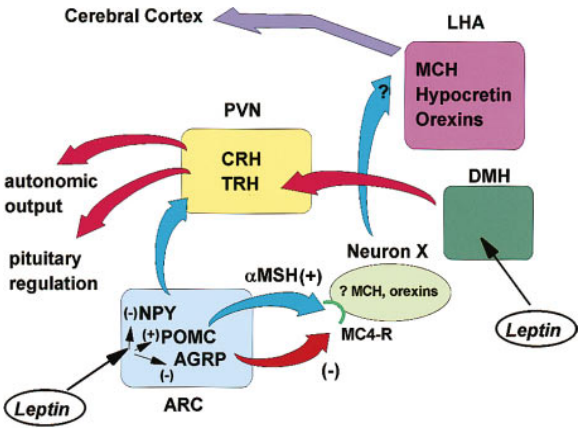

Figure 1. A Model of the Pathways of Leptin Action in the Hypothalamus

Through direct actions on cell bodies in the arcuate nucleus (ARC), leptin positively regulates POMC (α-MSH) and negatively regulates NPY and AGRP. α-MSH neurons project to MC4 receptor-expressing neurons of as-yet-uncertain chemical identity. AGRP-expressing neurons antagonize the α-MSH signal on these neurons. Directly or indirectly, this signal influences neurons in the lateral hypothalamus (LHA) that then influence hunger/satiety by mechanisms that may include long cortical projections involving MCH neurons. NPY projects to the paraventricular nucleus (PVN), which contains neurons expressing CRH and TRH (and other neuropeptides). The PVN influences anterior and posterior pituitary functions. The PVN also directly innervates autonomic preganglionic neurons, both sympathetic and parasympathetic. The dorsomedial hypothalmus (DMH) has neurons that are responsive to leptin and project to the PVN.

by increased linear growth and altered hair pigmentation. The disorder is caused by ectopic and unregulated expression of agouti, a protein normally restricted to hair follicles where it affects pigmentation by antagonizing melanocyte stimulating hormone (α-MSH). A breakthrough came with the demonstration that agouti antagonizes several members of the melanocortin receptor family (Lu et al., 1994), including the MC1 receptor in hair follicles (explaining the coat color) and MC4 receptors, which are largely restricted to the brain. This observation suggested that melanocortin receptor blockade in the brain could produce obesity, and has been followed by observations defining a system for metabolic regulation by two opposing ligands, one an agonist and the other an antagonist at the MC4 receptor (Figure 1).

Two results placed the MC4 receptor at a pivotal position in the central pathways for energy homeostasis. Targeted deletion of the MC4 receptor produced a syndrome of obesity (Huszar et al., 1997) similar to that of Ay mice, absent the pigmentary defect that required deregulated expression of agouti in skin acting on MC1 receptors. Additionally, the observations of decreased feeding after central administration of agonists (e.g., α-MSH) and the increased feeding after administration of a synthetic receptor antagonist (Fan et al., 1997) provide pharmacological evidence for the role of the MC4 receptor. What is the endogenous ligand for this receptor? Recent studies have produced a number of surprises. α-MSH, produced from proopiomelanocortin (POMC) precursors, appears to fit the role of agonist to decrease feeding. In the arcuate nucleus (which also expresses NPY), approximately 30% of the POMC neurons express the mRNA for the leptin receptor long form, and arcuate *POMC* mRNA expression is regulated positively by leptin (Thornton et al., 1997). Thus, one can envision a loop in which rising leptin (with obesity) drives increased arcuate POMC expression, which then projects α-MSH containing axons to MC4R-expressing cell bodies elsewhere in the hypothalamus, causing decreased food intake.

But the MC4 receptor pathway is not restricted to a single agonistic ligand, as a newly discovered neuropeptide acts as an antagonist on this same receptor. Agouti-related peptide (AGRP), also named agouti-related transcript (ART), is a homolog of agouti (Ollmann et al., 1997, and references therein) whose expression is largely restricted to the arcuate nucleus, where it is markedly increased in leptin deficient *ob*/*ob* mice. AGRP antagonizes α-MSH at the MC4-R (but not the MC1-R), and its forced ectopic expression in transgenic mice produces obesity closely resembling that of the MC4-R knockout mouse (Ollmann et al., 1997). Therefore, AGRP almost certainly acts as an antagonist at the MC4-R, as one member of an apparent yin–yang pair with α-MSH. The output from the feeding inhibitory MC4 receptor may be determined by the ratio of agonist (MSH) and antagonist (AGRP) at MC4 receptor neurons.

Does the MC4 receptor lay downstream of the pathway by which leptin induces some or all of its effects? Since MC4 receptor blockade or knockout produces obesity, but not reproductive failure or hypercortisolism as seen in leptin-deficient mice, it appears that MC4 receptor signaling is not required for the neuroendocrine effects of leptin deficiency. Is this pathway required for the satiety effects of elevated leptin? A downstream role for MC4 receptors in the satiety action of leptin is supported by two lines of evidence. First, Ay mice with MC4 receptor blockade develop obesity despite marked hyperleptinemia, and leptin administered peripherally or into the CSF has markedly reduced effect (Halaas et al., 1997). Second, pretreatment of rodents with an MC4 receptor antagonist acutely blocks the satiety-inducing action of leptin (Seeley et al., 1997). However, this view of the leptin-melanocortin axis has been challenged by results of a genetic cross between *ob*/*ob* and A^y/*a* mice. In these experiments, the A^y and *ob*/*ob* lesions were demonstrated to have additive effects on weight gain and serum insulin. These mice respond normally to exogenous leptin, suggesting that the leptin resistance of A^y/*a* mice requires, or is in some way a consequence of, the chronic leptin excess (Boston et al., 1997). Potential mechanisms might include down-regulation of leptin receptor or downstream signaling, or induction of antagonists by chronic leptin exposure. Clearly, the MC4 receptor is likely to mediate some of leptin's actions, but additional work will be required to define precisely what these are.

From the Mediobasal to the Lateral Hypothalamus

Heretofore, the molecular focus has been on the mediobasal hypothalamus, where the signaling form of the leptin receptor and neuronal cell bodies expressing NPY, POMC, and AGRP reside, all apparently under leptin control. But how do the signals from these sites impact the neural underpinning of hunger and satiety,

and by what route does the lateral hypothalamus play a role? Although the distance separating the arcuate nucleus and lateral hypothalamus is but a few millimeters, complex neural circuitry is likely involved, as information from leptin signaling is integrated with numerous other inputs relevant to nutritional homeostasis, including circadian signals and inputs from higher centers.

One potential link between the mediobasal hypothalamus and the lateral hypothalamus may involve melanocortin 4 receptors. These receptors are expressed in cells of the lateral hypothalamus (Mountjoy et al., 1994), but the chemical identity of these neurons is unknown. These neurons may provide an important link between the early steps of leptin action and the higher functions that underlie food-seeking behavior, since cells within the lateral hypothalamus (such as MCH neurons, see below) directly innervate higher cortical centers. Leptin-responsive cells in the dorsomedial hypothalamus may play a major role in transducing leptin's effects on autonomic output (Elmquist et al., 1998).

New Mediators in the Lateral Hypothalamus

The molecular mechanism for signaling from lateral hypothalamus to higher centers that regulate hunger and satiety has been obscure, but once again, genetic approaches have begun to clarify the chemical anatomy. By three distinct genetic approaches, five novel lateral hypothalamic neuropeptides that may play important roles in energy balance and obesity have been identified.

Melanin-Concentrating Hormone (MCH)

The first new actor was melanin-concentrating hormone (MCH) (Nahon, 1994, and references therein). Initially discovered in chub salmon pituitaries, salmon MCH circulates and regulates skin color by acting on melanosomes within pigmented skin cells. MCH lightens skin by inducing aggregation of melanosomes. α-MSH induces skin darkening in this system, by inducing dispersion of melanosomes. Thus, MCH and α-MSH are functional, though not pharmacological antagonists in the fish scale system.

The presence of MCH in mammalian hypothalamus was described in the mid-1980s, confined to a group of magnocellular neurons in the lateral hypothalamus and zona incerta. MCH neurons project to the nucleus of the solitary tract and the parabrachial nucleus, but are highly unusual in also having diffuse monosynaptic projections to the medial prefrontal cortex, leading to suggestions that MCH plays a role in complex integrative behaviors.

PCR-based differential display was employed to identify mRNA's that were expressed differentially in the hypothalamus of *ob*/*ob* mice compared to littermate controls, and MCH came out of this screen (Qu et al., 1996). Overexpression of MCH mRNA was seen in *ob*/*ob* mice; fasting increased expression both in control and *ob*/*ob* animals. Intracerebroventricular administration to rats stimulated feeding. It is of interest that MCH and α-MSH have opposing actions in rat brain, as they do in fish scales. This opposition is in models of auditory gating, grooming, and most recently feeding. However, unlike α-MSH and AGRP, which antagonize each other by acting on the same receptor, MCH has no affinity for known melanocortin receptors and acts via an as-yet-unidentified receptor.

Hypocretin

The second novel factor localized to the lateral hypothalamus is hypocretin, which is exclusively expressed in the lateral hypothalamus (de Lecca et al., 1998). Hypocretin was discovered through direction tag PCR subtraction, the goal of which was to identify mRNAs specifically expressed in hypothalamus. Hypocretin mRNA encodes two peptides that have homology to the gut hormone secretin, and neurons encoding hypocretin are located in an area that overlaps the distribution of MCH-containing neurons. Projections of these neurons are complex and extend into the arcuate nucleus, the septal nucleii and the basal forebrain, and the preoptic area. The signaling role of the hypocretins and whether they have any role in body weight regulation remain undefined.

Orexins

Finally, discovery of the two orexins and their receptors is reported in this issue of *Cell* (Sakurai et al., 1998). The effort that led to this discovery is a technical tour de force. Yanigasawa and colleagues sought ligands that bound to orphan G protein–coupled receptors. Using cell lines expressing 50 of these, they searched brain extracts for activation of calcium currents and succeeded in purifying and cloning cDNAs encoding two related peptides, orexins A and B, produced from a single protein precursor by proteolytic processing. The mRNA expressing the orexins is expressed in an extremely limited distribution within the lateral hypothalamus, with some expression within the testes. Remarkably, orexin expression increases with starvation, and central administration induces hyperphagia. Thus, quite rapidly, the number of peptides selectively expressed in the lateral hypothalamus has increased from none to five, and the number regulated by nutrition and capable of inducing hyperphagia has gone from none to three. It will be critical to determine whether MCH, orexins, and hypocretins are expressed in the same cells, and to map the projections of these cells throughout the brain. Potential functional interactions between these neuropeptides are also of great interest.

New Directions

Having identified an array of new neurochemicals that regulate appetite and energy homeostasis, much still remains to be done. First, there is little reason to suspect that the last of the important actors has been discovered, and so fishing expeditions will be trolling hypothalamic waters in search of more big trophy catches. New molecules in the pathway, such as MCH, orexins, and hypocretins will be deleted and overexpressed, and genetic models will be combined to produce models of gene interactions in vivo. A role of these genes in human obesity will also be sought. Since monogenic human obesities are likely to be uncommon, it will be necessary to probe interactions between the central pathways that respond to leptin and other signals involved in energy homeostasis. For example, what are the adaptations to knockout or overexpression of individual neuropeptides and receptors, and how do these potential adaptations interact with genetic background and environmental conditions such as diet and stress.

The foregoing reminds us that, for humans at least, decisions to eat or not (or more precisely what to eat

and when to stop) are highly complex, residing at the fuzzy interface between free will and physiology. We eat for many reasons, including those that are hedonic, those that emerge from psychic conflicts, and those that relate to basic survival. Our new knowledge of the chemical anatomy of the lateral hypothalamus is a long way from reducing these complex issues to simple synaptic equations. On the other hand, since massive obesity in humans may result from defective signaling by a single receptor (e.g., the leptin receptor) (Montague et al., 1997), we are reminded that the genetic roots of hunger and satiety in humans run deep. The continued pursuit of these factors will be productive for years to come.

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