Melanocortin pathway: animal models of obesity and disease

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INTRODUCTION

The last decade has witnessed significant advances in our understanding of energy homeostasis and regulation of body weight. With the discovery of the adipose hormone leptin, investigators quickly began to unravel the neural circuits responsible for mediating the leptin signal in the brain [1-3]. Initial studies localized the hypothalamic nuclei that expressed leptin receptors and those that were activated by peripheral leptin administration [4-6]. The identity of the neuronal populations that respond to leptin and exert central control over processes fundamental to the intake, use, and storage of body fuels are also being elucidated. In particular, the cloning of the agouti gene [7, 8] and the discovery of its mechanism of action [9-11] led to the identification of a set of neuronal pathways that we will refer to as the melanocortin system. In general, the melanocortin system can be defined as the hypothalamic and brainstem neurons expressing pro-opiomelanocortin (POMC), the hypothalamic neurons co-expressing neuropeptide Y (NPY) and the melanocortin antagonist agouti-related protein (AGRP), and the neurons downstream of these systems. POMC neurons are leptin responsive and may mediate many of its feedback effects [12]. In addition, the melanocortin system has effects on weight and metabolism that are independent of leptin feedback and that leptin-deficient animals with disrupted melanocortin signaling remain sensitive to leptin administration [13, 14]. In general, POMC neurons represent a logical target for leptin and other cytokine-mediated feedback on feeding behavior and metabolic control, and recent data suggest a role in anorexia and cachexia as well [15, 16].

THE HYPOTHALAMIC ADIPOSTAT

In addition to the melanocortin system, other neuronal pathways have also been identified as targets of leptin action within the hypothalamus. In particular, neuropeptide Y (NPY) is produced in hypothalamic nuclei known to regulate appetite and metabolism, and NPY is a potent orexigen when injected centrally [29, 31-35]. Leptin deficiency results in a significant upregulation of NPY expression while leptin administration causes a decrease in expression [36, 37]. Furthermore, genetic deletion of NPY in leptin-deficient animals results in a significant decrease in their degree of obesity [17]. In general, it appears that NPY synthesis and secretion are upregulated in most models of energy deficiency or increased metabolic demand [18, 19]. Thus, an abnormality in NPY expression is one potential mechanism for the anorectic effects of leptin. Recently, we have demonstrated that NPY neurons located in the arcuate nucleus are inhibited by leptin administration, lending further support for the role of NPY in mediating the normal feedback effects of leptin [12].

In contrast to the stimulatory effects of NPY, proopiomelanocortin (POMC) neurons are thought to provide an important tonic inhibition of food intake and energy storage, primarily via production and release of alpha-melanocyte stimulating hormone (alpha-MSH) from the POMC precursor. Alpha-MSH binds to central melanocortin receptors (including the type 4 melanocortin receptor, MC4-R) where it acts to inhibit food intake [9]. POMC neurons in the arcuate nucleus express the leptin receptor and MC4-RKO mice are leptin resistant, leading several investigators to propose that melanocortin neurons mediate the anorectic effects of elevated leptin [20,
MELANOCORTINS AND OBESITY

While many factors contribute to the development of obesity in humans, there are many features that occur to some extent in the majority of obese individuals. Moderate to severe hyperphagia is coupled with an efficient metabolism to begin the process of energy storage in the form of fat. As individuals age, they tend to continue to accumulate fat, and become increasingly insulin resistant with age. Obese children and adults generally have increased lean body mass in addition to fat mass, but otherwise have normal endocrine function. The majority of these features are recapitulated in the dominantly inherited lethal yellow mouse (Ay/–). These mice are hyperphagic, have an efficient metabolism, and develop insulin resistance as they age [24–27]. These animals have normal reproductive and adrenal axes [28], and increased somatic growth [29], features that are all found in the development of obesity in humans. The basis for the obesity in the lethal yellow mouse is its ectopic expression of agouti, a paracrine factor normally involved in the regulation of pigmentation [7]. The ectopic agouti peptide mimics the normal function of AGRP in the hypothalamus in that it binds to the MC4-R and blocks its function. In general, it appears that stimulates MC4-R signaling will lead to decreased food intake and increased metabolic rate, while antagonism of this receptor has the opposite result [9]. This activity is particularly obvious in the MC4-RKO animal, which recapitulates all of the primary metabolic, growth, and behavioral phenotypes of the agouti obesity syndrome [11].

Since the discovery of the importance of melanocortins to the regulation of metabolism and appetite in rodents, several investigators have examined the potential role of melanocortin signaling in the development of human obesity. Specifically, families with rare deleterious mutations in the POMC gene have been described in whom the defect results in a syndrome of red hair, ACTH deficiency, and obesity [30], and heterozygous mutations in the MC4-R have been reported to be associated with common pediatric obesity [31, 32]. Another group has demonstrated strong genetic linkage of obesity to the POMC locus on chromosome 2p21, although no defects in the coding region of the gene were found [33]. Collectively, these data argue strongly that the melanocortin system plays a role in the regulation of energy homeostasis in mice and humans.

The finding that deletion or blockade of components of the melanocortin system will lead to obesity does not tell us the specific physiological role for this system in the normal animal. Recently, we have begun to elucidate the unique metabolic defects found in MC4-R deficient animals, and have concluded that these animals do not have a simple gross defect in matching energy intake with energy expenditure. Indeed, these animals respond normally to many metabolic challenges including food restriction or exposure to cold environments [34]. In a food restriction paradigm the MC4-RKO and wild-type control animals lose weight at a similar rate, and return to baseline weight after refeeding with identical kinetics. In a cold environment, KO and control animals both compensate for increased energy output with a comparable amount of hyperphagia and weight gain. In contrast, our experiments show a marked defect in the ability of the MC4-RKO animal to respond to acute changes in nutrient intake. When normal mice are switched from normal low fat chow to a moderate fat chow with higher caloric density, they will respond with an appropriate decrease in food intake to maintain an isocaloric intake. They will also show increased thermogenesis and increased activity in response to this diet change, all of which prevents abnormal weight gain on this diet. In contrast, MC4-RKO animals actually increase their food intake on the high-fat food, and fail to show any changes in thermogenesis or activity. Thus, these animals undergo a dramatic increase in the rate of weight gain when placed on the moderate fat chow. Leptin deficient animals responded normally in this paradigm, indicating that this function of melanocortin receptors does not depend on leptin feedback.

MELANOCORTINS AND CACHEXIA

The role of nutrition and balanced metabolism in normal growth, development, and maintenance of body mass is well known. Children affected with either acute or chronic diseases often show disorders of nutrient ba-
lance while others have no obvious organic disease and are given the observational diagnosis of failure to thrive. Adults may lose dramatic amounts of weight when ill, even when interventions such as parenteral nutrition are used. In some cases, a devastating state of malnutrition known as cachexia arises, brought about by a synergistic combination of a dramatic decrease in appetite and an increase in metabolism of fat and lean body mass. This combination is found in a number of disorders including cancer, cystic fibrosis, AIDS, rheumatoid arthritis, and renal failure [35]. The severity of cachexia in these illnesses is a primary determining factor in both quality of life, and in eventual mortality [35, 36]. Indeed, body mass retention in AIDS patients has a stronger correlation with survival than any other current measure of the disease [37]. This has led to intense investigation of cachexia and the proposal of numerous hypotheses regarding its etiology. At this point, most authors suggest that cytokines released during inflammation and malignancy act on the central nervous system to alter the release and function of a number of key neurotransmitters, thereby altering both appetite and metabolic rate [19, 35, 38, 39].

We observed that stimulation of melanocortin receptors produced many of the metabolic features seen in cachexia, and proposed that cytokine feedback may be mediated, in part, by POMC neurons. To test this hypothesis, we administered a purified product found in the cell wall of gram negative bacteria known generically as lipopolysaccharide (LPS) to wild type and MC4-RKO animals. This compound is known to reliably produce anorexia and increased metabolic rate in wild type mice due in large part to the potent release of numerous cytokines [40-43]. Remarkably, the MC4-RKO animals were resistant to the anorexigenic and metabolic effects of LPS administration and also showed decreased illness behavior during the experiment [15]. Additionally, we have also demonstrated that in young, rapidly growing mice, the weight loss that accompanies LPS-induced illness can be reversed, allowing the animals to continue to follow a normal growth curve. Our data are consistent with those of Tatro et al. who showed that MC4-RKO mice resist the inhibition of locomotion produced with central IL1-β administration [44]. In another recent study, Huang et al. investigated the impact of central administration of α-MSH or the melanocortin receptor subtype3/subtype4 antagonist SHU-9119 on LPS-induced anorexia and fever in rats [16]. In this study, the investigators found a significant potentiation of the suppressive effects of LPS on food intake with administration of α-MSH, and a reversal of LPS-induced anorexia with SHU-9119 administration.

Clearly central melanocortins play a role in mediating the acute anorexia and fever induced by LPS. Our next question was whether these observations could be extended to demonstrate a role of melanocortin receptors in transducing the prolonged metabolic derangement observed in experimental cancer. Many different tumor types have been studied and it is a common finding that tumor-bearing animals die from cachexia and exhaustion of metabolic fuels, rather than from metastasis or infection [45-48]. Our first experiments demonstrated that hypophagia and carcass weight loss induced by sarcoma growth can be both reversed and prevented by administration of the endogenous MC3/MC4 antagonist, AGRP [15]. Prevention of tumor-induced hypophagia with early and repeated AGRP injections resulted in a maintenance of normal food intake, and this enhancement of feeding was much greater than the relative hyperphagia observed in the sham-tumor implanted animals. Tumor growth was unaffected by the AGRP treatment, demonstrating the specificity of these results. The finding that central melanocortin blockade attenuates cancer cachexia was confirmed in MC4-R KO mice. The MC4-RKO animals had normal feeding and growth even when bearing a carcinoma that produced classic cachexia in wild-type control animals. In these animals, serial dual energy X-ray absorptometry scans confirmed that the tumor bearing MC4-RKO animals continue to accumulate both lean mass and fat mass in the face of tumor growth. The wild-type animals lost both lean mass and fat mass under identical conditions. These data clearly indicate that the hypothalamic MC4-R plays a role in transducing cachexigenic stimuli from the periphery. However the contribution of the MC3-R (which is also blocked by AGRP administration) is not known. Furthermore, the mechanism whereby melanocortin signaling is altered during illness is not known, nor have we identified the neural targets downstream of these receptors. Overall, a better understanding of central melanocortin physiology during acute and chronic disease may eventually lead to the development of specific antagonists that will be useful in treating cachexia.

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