AgRP neurons: The foes of reproduction in leptin-deficient obese subjects

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Evolutionarily, the ability to regulate energy balance and reproduction in parallel is critical, because reproductive success will only occur when sufficient energy supplies are available. In periods when energy stores are depleted, reproduction is switched off in an attempt to save energy to optimize survival for subsequent reproductive success. The cellular mechanisms involved in the fine coordination of energy balance and reproduction are largely unknown. In PNAS, Wu et al. (1) shed light on a previously unsuspected neuronal population that appears to be fundamental for the coupling of energy deficit with impaired reproduction.

Energy Availability and Reproduction

To perpetuate the species, individuals must maintain an adequate energy balance, which will ultimately allow reproduction. The proper energy homeostasis must be sensed by the whole body to promote the proper endocrine and behavioral switches in support of reproductive success. One mechanism by which the organism transmits information about energy stores is through circulating leptin. Leptin is released by the adipose tissue, and its levels are proportional to the amount of fat (2, 3). Increased leptin levels feed back to tissues to decrease energy intake and deposition and to increase energy expenditure. In such situations, reproduction is the best to occur, because energy is then available to develop a new organism. On the other hand, during depleted energy states (e.g., fasting, malnutrition) that lead to fat depletion, leptin levels are low and reproduction is turned off. One of the phenotypes of chronic fasting or malnutrition is hypothalamic hypogonadism, which is promptly reversed on recovery of energy stores.

Circulating leptin deficiency is a naturally occurring mutation in both rodents (4) and humans (5) that leads to a complex phenotype combining obesity, diabetes, and infertility (including hypothalamic hypogonadism). This phenotype is not permanent, and it can be reversed by chronic treatment with recombinant leptin (6), indicating that developmental abnormalities caused by leptin deficiency are insufficient to interfere with normal leptin regulation of reproduction and energy homeostasis in the adult.

Leptin and Hypothalamic Regulation of Homeostasis

The brain is a critical player in the regulation of whole-body homeostasis, and leptin is acting on the brain to affect integrative physiology. One population of neurons responsive to circulating leptin is those that express agouti-related peptide (AgRP), in addition to neuropeptide-Y (NPY) and GABA (7, 8), in the arcuate nucleus of the hypothalamus. These neurons are mandatory to respond behaviorally to low energy levels to promote appetite (9–11). When ablated in adult mice, animals die because of lack of interest in energy uptake (12, 13). The effects of elimination of these neurons on these processes have been attributed to the inhibitory neurotransmitter GABA (14). Wu et al. (1) use the same animal model to target ablation of the AgRP neurons in adult mice to ask whether these neurons are important for the phenotypes that emerge in leptin-deficient mice. In certain cases, ablation of AgRP neurons in leptin-deficient (*ob*/*ob*) mice did not lead to death, despite a prolonged period of starvation. Specifically, only mildly obese leptin-deficient mice survived AgRP-ablation; mice heavier than 40 g lost weight, had decreased body temperature, and became moribund. Those *ob*/*ob* mice that survived 2 wk of almost complete starvation gradually recovered and reached the body weight, food intake, and glucose metabolism of control mice. Most remarkably, however, these animals became fertile.

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AgRP Neurons and Reproduction

Conceptually, Wu et al. (1) are supported by previous reports showing that NPY/AgRP neurons influence the metabolic and reproductive phenotype of *ob*/*ob* mice. Leptin-deficient mice have hyperactivation of the NPY/AgRP neurons (15), similar to mice in a fasting state (7). Chronic administration of NPY in the brain of normal animals mimics the phenotype of leptin deficiency, including decreased fertility (16–18). Despite these effects of NPY, KO mice for the *npy* gene have a normal metabolic phenotype (19). However, deletion of NPY in leptin-deficient mice (double-KO mice) partially restored fertility and promoted mild improvement in metabolic phenotype (20). These effects on fertility seem to be dependent on Y4 receptor (21). It is worth noting that ablation of AgRP neurons in neonates does not influence fertility in adult mice (12, 22). It also remains to be seen whether neonatal ablation of AgRP neurons in *ob*/*ob* mice will influence metabolism and reproduction in adult mice.

The effects of AgRP ablation rescuing fertility in *ob*/*ob* mice are remarkable because leptin was thought to play a crucial role in puberty and subsequent reproductive success. Leptin is clearly not a player in restoring fertility in these animals. Intriguingly, similar to leptin, the primary gonadal steroid hormone estrogen also reduces food intake and body adiposity and increases energy expenditure even in the complete absence of circulating leptin in *ob*/*ob* mice (23). The central effect of estrogen in the regulation of reproduction is directly related to reproductive hormone cycles. The actions of estrogen on the hypothalamic gonadotropin-releasing hormone (GnRH) neuronal network are required to trigger the episodic release of GnRH, which leads to a pulsatile pattern of luteinizing hormone (LH) secretion. Reproduction is critically coordinated by the hypothalamic anteroventral periventricular nucleus and the preoptic area, where GnRH neurons reside. GnRH neurons are the final output of a network that integrates environmental and hormonal cues to regulate the secretion of reproductive hormones; they

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are inhibited by negative energy balance. The stimulatory effect of estrogen triggers the episodic release of GnRH and induces a pulsatile pattern of LH secretion. Leptin pretreatment prevents fasting-induced reduction of the activities of GnRH neurons, suggesting that the knowledge of preexisting body energy stores, indexed by leptin levels, is crucial for GnRH neuron function.

It is likely that the AgRP neurons and the GnRH neurons are either directly or indirectly connected (24) and that this circuitry dictates the reproductive phenotypes observed in several reports, including the one in PNAS (1). An intriguing question is how hypothalamic circuitry adapts to the lack of AgRP neurons reversing infertility in *ob*/*ob* mice in the complete absence of leptin. It is possible that synaptic plasticity, as already shown in the melanocortin system of *ob*/*ob* mice (15, 23), may be implicated in the adaptation of these mice to the lack of AgRP neurons.

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