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To subjugate NPY is to improve the quality of life and live longer

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Abstract

The interactive network of Neuropeptide Y (NPY) and cohorts is necessary for integrating the hypothalamic regulation of appetite and energy expenditure with the endocrine and neuroendocrine systems on a daily basis. Genetic and environmental factors that produce an insufficiency of leptin restraint on NPY and cognate receptors deregulate the homeostasis to engender various life-threatening risk factors. Recent studies from our laboratory show that neurotherapy consisting of a single central administration of recombinant adeno-associated virus vector encoding the leptin gene can repress the hypothalamic NPY system for the lifetime of rodents. A major benefit of this stable tonic restraint is deceleration of pathophysiologic sequelae that shorten life span. These include suppression of weight gain, fat accumulation, circulating adipokines, amelioration of major symptoms of metabolic syndrome, improved reproduction and bone health. Thus, sustained repression of NPY signaling in the hypothalamus by leptin transgene expression can improve the quality of life and extend longevity.

INTRODUCTION

Since the demonstration of the potent appetite stimulating effects of neuropeptide Y (NPY) and cohorts in 1984 (18), these signaling molecules have been at the forefront of the intense research directed toward deciphering the dialogue between peripheral signals and hypothalamic circuits that orchestrate ingestive behavior in mammals (8,30,36,39,40,64). In addition to a pivotal role in hypothalamic integration of energy homeostasis, the hypothalamic NPY network has since been shown to play a role in neuroendocrine control of reproduction and lactation, fluid balance, growth, stress, temperature, general body metabolism and bone health (1,12,15,31,41,45,46,48,62). It is now obvious that disruption in any one or more loci in the hypothalamic network of NPY and cohorts inflicted by environmental or genetic factors leads to several metabolic and neuroendocrine diseases that adversely affect the quality of life and life span (1,4,24–26,30,32,47). The focus of this review is to summarize our recent efforts to characterize the potential health benefit of experimentally imposed lifetime restraint on hypothalamic NPYergic signaling by leptin, the endogenous adipocyte hormone that is intimately involved in the hypothalamic integration of energy and neuroendocrine homeostasis (2,3,9,10,12–15,19–21,23,33,37,38,42,48,60,61).

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Functional Neuroanatomy of hypothalamic NPY

NPY expressing neurons in the arcuate nucleus (ARC), dorsomedial hypothalamus (DMH) and brain stem (BS) innervate various hypothalamic sites implicated in integration of energy balance (30,36,39,40,64). These NPY neurons also coexpress various neurotransmitters. Whereas the ARC NPY subpopulation coexpresses the neuropeptide agouti-related peptide (AgRP) and the amino acid γ -aminobutyric acid (GABA), the BS subpopulation coexpresses adrenergic transmitters, epinephrine and norepinephrine. A dynamic interplay of these neurotransmitters in the ARC and paraventricular nucleus (ARC-PVN) neuroaxis is believed to propagate periodic appetitive behavior (18,36,39,40,60,65). A mapping of various aspects of NPY neurosecretion pattern (i.e. synthesis in ARC perikarya, storage and release in the terminal field in the ARC-PVN axis) showed the episodic rise and fall in NPY release to be temporally correlated with initiation and termination of the appetitive drive (36,39,40). Knowledge of this tight temporal relationship between neurosecretion and behavior greatly assisted in delineating the precise roles of afferent hormonal signals from the periphery, such as leptin from white adipose tissue (WAT), ghrelin and peptide YY (PYY₃₋₃₆) from gastrointestinal tract and insulin from pancreas β -cells, in the release of NPY antecedent to meal initiation (3,4,8,22,35,42,44,52,53,55,60,64,65). Photoperiodic cues and the time of food availability also interact in modulating the periodic antecedent NPY neurosecretion in the ARC-PVN axis (36,39,40,55,60,65). NPY expressing neurons in the DMH and BS are goaded into action to cope with those physiological and environmental challenges that demand increased energy, such as lactation or derangements in afferent neural and hormonal feedback signals to the hypothalamus (36,39–41). In addition, the hypothalamic NPY network exerts control on the hypothalamic anorexigenic melanocortin and the orexigenic orexin and melanin concentrating hormone (MCH) signaling to NPY Y₁ and Y₅ receptor expressing targets in the ARC-PVN axis (2,30,36,39,40,64).

During the course of these investigations, it became apparent that environmental insults and genetic derangements that disrupt information flow by causing either low or high abundance of NPY in the ARC-PVN axis, always confer hyperphagia and never hypophagia (24–26,32, 36,39,40). This increased energy intake response coalesces into various pathophysiological afflictions that negatively impact the quality of daily life and longevity, namely obesity and the metabolic syndrome cluster of disorders including glucose intolerance, hyperinsulinemia, atherosclerosis, cardiovascular ailments and fatty liver, and disruption in reproduction, fluid homeostasis, temperature control and energy expenditure (12,15,35,37–39,42).

Is NPY a naturally occurring appetite transducer?

Converging experimental evidence of antecedent increases in NPY release in the ARC-PVN axis correlated temporally with the initiation of periodic meal patterns, NPY hypersecretion with fasting coincident, and suppression of appetitive drive following experimental attenuation of NPY availability at Y₁ and Y₅ receptors, either pharmacologically or by physiological and genetic manipulations, implied that NPY is a naturally occurring appetite transducer in the brain (36,39,40). Results from diverse molecular and genetic paradigms are consistent with the implication that NPY, in concert with co-expressed orexigenic AgRP, GABA and adrenergic transmitters, constitutes an obligatory orexigenic signaling modality that is intimately involved in propagation of the timely appetitive drive under the direction of photoperiodic and hormonal cues (7,11,27–29,47,49–51,54,56–59). Additional recent disclosures that the hard wiring for the timely operation of this interplay is established during postnatal development have put the notion that NPY is a physiological appetite transducer on firm footing (11,28–30,49,64).

Is it possible to experimentally subjugate the hypothalamic network of NPY and cohorts?

In view of the underpinnings that 1) NPY is a physiological appetite inducer and 2) that disruptions in information flow in the hypothalamic network of NPY and cohorts causing either

overabundance or low abundance of NPY engender only hyperphagia, obesity, decrease in energy expenditure, and a multitude of metabolic diseases, it is highly pertinent to investigate the feasibility of experimentally subjugating NPY signaling in order to avert or slow the onset and progression of this spectrum of ailments. Pharmacologic approaches that either attenuate NPY synthesis and release or antagonize NPY Y₁ and Y₅ receptors in the ARC-PVN axis have been evaluated *in extenso* (39,40). In general, NPY receptor antagonists, despite relatively poor solubility and penetration across the blood-brain barrier, are transiently effective in suppressing food intake and body weight gain (39,40). The efficacy and safety of newer low molecule NPY receptor antagonists, which can readily and safely penetrate the blood-brain barrier and curb weight gain over long periods, remain to be ascertained.

An alternate approach, which delivers endogenous signals to suppress appetite and decelerate weight gain for extended periods by targeting the hypothalamic network, is a promising modality to circumvent the shortcomings of pharmacological interventions (34,37,43). Leptin, a product of the *ob* gene, is produced by WAT and non-adipocytes tissues, including stomach and hypothalamus (30,36,55,63,65). It is a pleiotropic hormone in the periphery and plays an important role in the hypothalamic integration of energy homeostasis (37,38). In general, leptin restrains the hypothalamic interactive appetite regulating network, and simultaneously augments energy expenditure (36,37). These effects of leptin are mediated by leptin receptor expressing orexigenic NPY, orexin and MCH neurons, and anorexigenic proopiomelanocortin (POMC) and steroidogenic factor-1 expressing neurons (4,30,36,64). Insufficiency of leptin, specifically in the hypothalamus, decreases the leptin restraint and restoration of leptin availability by peripheral or central administration of leptin readily reinstates this restraint on the interconnected NPY network (36,37). Seemingly, sustenance of an optimal leptin restraint on the network of NPY and cohorts is crucial for hypothalamic integration of energy homeostasis for lifetime (36,37).

Because leptin levels decrease in the brain with increasing adiposity due to diminution in leptin transport from the periphery compounded by inadequate supply of locally produced leptin, (5,6,16), and because central replacement of leptin readily reinstates energy homeostasis despite hyperleptinemia in obese rodents, we proposed that hypothalamic leptin insufficiency is one of the causative mechanisms for age-related as well as dietary obesities, which are invariably accompanied by increased incidence of metabolic diseases (14,19–21,23,36,37,52, 53,55). To verify this hypothesis, it is essential to address the following question: can a lifelong leptin restraint on the hypothalamic interconnected network of NPY and cohorts be imposed experimentally?

Repression of hypothalamic NPY by central leptin-gene therapy

Among the various viral vectors available for delivery of target genes for life-long transduction of proteins in neurons, the non-replicative, non-pathogenic and non-immunogenic recombinant adeno-associated virus vector (rAAV) was employed to validate the central leptin insufficiency hypothesis (17,33,34,43). rAAV encoding leptin gene (rAAV-lep) readily transduced leptin *in vitro* and *in vivo* in leptin-deficient *ob/ob* mice (19). Injection of rAAV-lep either intracerebroventricularly (icv) or to various hypothalamic and extrahypothalamic sites of rats and mice was highly efficient in restraining the age-related and high fat diet-induced weight gain for the duration of the experiments ranging from a few weeks to nearly two years (2,3,9, 10,13,14,19–21,23,44,60,61). This enhanced availability of bioactive leptin selectively in the hypothalamus conferred the anticipated responses from the leptin receptor expressing orexigenic and anorexigenic neuronal targets. NPY mRNA expression was suppressed and POMC mRNA expression in the ARC was simultaneously augmented (2,9,10,12,19–21). However, quite unexpectedly, icv rAAV-lep injection failed to modify AgRP mRNA expression in NPY coexpressing neurons. Conceivably, sustained leptin delivery in the

hypothalamus can selectively and stably restrain NPYergic signaling to the Y₁ and Y₅ receptor expressing targets in the PVN and Y₁ receptor expressing POMC targets in the ARC without the participation of AgrP in this interplay (37,38).

Global consequences of a stable lifetime repression of hypothalamic network of NPY and cohorts by leptin-gene therapy

A spectrum of beneficial effects, as enumerated below, were observed by maintaining a tonic restraint on hypothalamic NPYergic signaling with central leptin gene therapy in rodents.

1. Suppression of the gradual age-related and high fat diet-induced accelerated weight gain and adiposity in rats and mice and leptin-mutant, *ob/ob* mice for extended periods. There was no evidence of development of resistance to ectopic leptin in the hypothalamus (2,3,10,14,21,23,37).
2. Diminution of fat deposition engendered by increased energy expenditure, with or without a voluntary decrease in food intake, was apparently responsible for the long lasting suppression of weight gain (10,20,21,23,37).
3. The age-related as well as high fat diet-induced hyperglycemia, hyperinsulinemia and dyslipidemia were abolished concomitant with insulinopenia for the duration of the experiments (2,14,20,23,42,48,52,60,61).
4. A sustained restraint on hypothalamic NPYergic signaling also increased insulin sensitivity along with euglycemia and abolition of dyslipidemia not only in normal rats and mice, but also in hyperinsulinemic leptin mutant *ob/ob* mice and insulinopenic Akita mice. A clamp both on post-prandial and basal rhythmic insulin secretion, either alone or in combination with reduced fat mass, seems to confer insulin hypersensitivity in these rodents (12,60,61).
5. Abrogation of the disease cluster of metabolic syndrome in response to repression of hypothalamic NPYergic signaling was also concurrent with leptinopenia. This observation, together with the fact that central rAAV-lep injection enhanced leptin availability in the hypothalamus without any leakage to the periphery, clearly identified the hypothalamus as the site of inhibition by leptin of the basal rhythmic as well as post-prandial and high fat diet-induced insulin hypersecretion. Thus, it is possible to selectively activate hypothalamic receptors for a sustained increase in insulin sensitivity, thereby preventing the development of glucose intolerance and type 2 diabetes (12,20,52,60,61).
6. Central leptin gene therapy conferred benefits on the hypothalamic regulation of neuroendocrine and endocrine functions. Leptin gene therapy increased testis weight, improved bone health, and suppressed fatty liver. Conceivably, these benefits were a consequence of blockade of leptin insufficiency-induced disintegration of the hypothalamic control on various physiological and neuroendocrine functions (12, 15,37).
7. Maintenance of leptin sufficiency reduced mortality rate and aging. Several metabolic and endocrine biomarkers of aging were absent after a single icv injection of rAAV-lep in leptin deficient *ob/ob* mice, including glucose intolerance, hyperinsulinemia, dyslipidemia and diminution in circulating levels of ghrelin and insulin-like growth factor-1, and altered secretion of proinflammatory cytokines and adipokines (2,10, 12,15,37,53,60,61). Repression of these deleterious metabolic biomarkers of aging, in aggregate, seemed to reduce mortality and double the lifespan of these mice (15, 37).

CONCLUDING REMARKS

We summarize evidence to show that a breakdown in the operation of the hypothalamic network of NPY and cohorts due to insufficiency of leptin restraint leads to imbalance in energy intake and expenditure which, in turn, culminates in obesity, metabolic syndrome and shortened lifespan. Reinstatement of the optimal level of leptin restraint on NPYergic signaling can prevent these varied afflictions, improve the quality of daily life and extend longevity. Consequently, we propose that future interventions, such as those that involve leptin gene transfer or employ novel long-acting leptin mimetics to efficiently prevent the consequences of disruption in NPYergic signaling, are potentially novel anti-aging therapies.

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