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Neuropeptide Y in noradrenergic neurons induces obesity in transgenic mouse models

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ABSTRACT

Neuropeptide Y (NPY) in noradrenergic neurons plays an important role in modulating the release and effects of catecholamines in a prolonged stress response. Among other functions, it controls energy metabolism. Transgenic expression of *Npy* in noradrenergic neurons in mice allowed showing that it is critical for diet- and stress-induced gain in fat mass. When overexpressed, NPY in noradrenergic neurons increases adiposity in gene-dose-dependent fashion, and leads to metabolic disorders such as impaired glucose tolerance. However, the mechanisms of obesity seem to be different in mice heterozygous and homozygous for the *Npy* transgene. While in heterozygous mice the adipogenic effect of NPY is important, in homozygous mice inhibition of sympathetic tone leading to decreased lipolytic activity and impaired brown fat function, as well as increased endocannabinoid levels contribute to obesity. The mouse model provides novel insight to the mechanisms of human diseases with increased NPY due to chronic stress or gain-of-function gene variants, and a tool for development of novel therapeutics.

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1. Introduction

Neuropeptide Y (NPY) is abundantly expressed in various types of neurons in central as well as peripheral nervous systems. It is often co-localized in the same neurons with other neuropeptides or neurotransmitters that in interaction control various functions (McDonald and Pearson, 1989). In catecholaminergic neurons, NPY is co-localized with noradrenaline and adrenaline (Sawchenko et al., 1985), and takes part in the stress-response of sympatho-adrenal and central noradrenergic systems. Here we review recent work using transgenic expression of *Npy* under dopamine-beta-hydroxylase (DβH) promoter in mice elucidating the long-term effects of NPY in adrenergic and noradrenergic neurons of the brain and sympathetic nervous system (SNS) especially on energy homeostasis.

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2. NPY in noradrenergic neurons

In sympathetic nerve endings, NPY is co-localized with noradrenaline. It is stored in large dense-cored vesicles, where it is released together with noradrenaline after a prolonged sympathetic stimulus such as physical exercise or cold exposure (Ekblad et al., 1984; Fried et al., 1985; Potter, 1988). In contrast, small dense-cored vesicles containing only noradrenaline respond more rapidly for an acute sympathetic stimulus. Similarly in chromaffin cells of the adrenal medulla, NPY is co-localized and co-released with noradrenaline and adrenaline (Varndell et al., 1984). In the brainstem, NPY co-localizes with either adrenaline or noradrenaline, and the most prevalent catecholaminergic neurons expressing NPY are A1/C1 neurons, locus coeruleus (LC) and the nucleus tractus solitarius (NTS) (Chronwall et al., 1985; Everitt et al., 1984).

The role of NPY in catecholaminergic neurons is bimodal. On one hand, it acts on target tissues by augmenting or prolonging the effects of adrenergic stimulation. For instance, hypoglycemia-induced feeding requires the release of both NPY and noradrenaline (Li et al., 2009). On the other hand, NPY regulates the synthesis and release of noradrenaline and adrenaline. Some evidence suggests that NPY increases the release of catecholamines (Renshaw et al., 2000), but most work supports a tonic inhibitory role for NPY (Cavadas et al., 2006; Wang et al., 2013). This inhibitory action of NPY was recently suggested to play a critical role in maintaining the secretory capacity of adrenal gland in repeated stress (Wang et al., 2013).

NPY plays an important role in coping different kinds of stresses. The behavioral and cardiovascular effects of NPY under stressful situations have been widely addressed (Abe et al., 2010; Heilig, 2004). Recently, the effects of NPY on energy metabolism have been studied in mouse models of stress-induced NPY release, and NPY overexpression in noradrenergic and adrenergic neurons (Kuo et al., 2007; Ruohonen et al., 2008; Vähätalo et al., 2015b; Zhang et al., 2014). These studies show that whereas acute sympathetic stress response leads to a rapid release of substrates from energy stores for immediate use, increased long-term release of NPY leads to increased energy storage as fat. This may be beneficial in prolonged stress in a harsh environment, but in modern societies with high-calorie foods easily available, it may lead to obesity and metabolic diseases.

3. Obesity and related co-morbidities in mice overexpressing NPY in noradrenergic neurons

The effects of NPY overexpression in noradrenergic and adrenergic neurons have been elucidated using a mouse model overexpressing (OE) *Npy* under *DβH* gene promoter (OE-NPY$^{D\beta H}$ mouse model) maintained in C57Bl/6N background strain. The OE-NPY$^{D\beta H}$ mouse has been studied as heterozygous (Ruohonen et al., 2008, 2009a, 2009b) and homozygous for the transgene (Vähätalo et al., 2015a, 2015b). The phenotype of the OE-NPY$^{D\beta H}$ mouse resembles the human metabolic syndrome with increased adiposity, hepatosteatosis, hypercholesterolemia and impaired glucose tolerance.

OE-NPY$^{D\beta H}$ mouse develops increased adiposity that is enhanced with age and with increased gene copy number. White adipose tissue (WAT) mass is increased by 1.2-fold in heterozygous and 1.8-fold in homozygous male mice in comparison with their wild-type (WT) controls. Lean mass in homozygous OE-NPY$^{D\beta H}$ mice is slightly declined, and a significant increase in body weight is evident around 4 months of age. Obesity is evident in both male and female mice when fed on regular laboratory chow. In contrast, when fed on Western-type high-fat, high-sucrose diet, the female OE-NPY$^{D\beta H}$ mice gain significantly more weight and fat mass in comparison with WT female mice, whereas there is no difference between the genotypes in the males (Ruohonen et al., 2012).

Lipids start to accumulate to other tissues besides WAT even when the mice are fed on chow. In homozygous OE-NPY$^{D\beta H}$ mice, increased brown adipose tissue (BAT) weight is evident already in early adulthood. The brown adipocytes start to resemble white adipocytes with single, large lipid droplets (Vähätalo et al., 2015b). The genotype difference in BAT is present also in OE-NPY$^{D\beta H}$ females fed on Western diet, indicating that *Npy* overexpression overrides the resistance of WT C57Bl/6N females to high-energy diet (Ruohonen et al., 2012).

Another critical site of excess lipid accumulation is the liver. Increased triglyceride stores in the liver are evident in heterozygous mice (Ruohonen et al., 2008) and hepatosteatosis deteriorates with age in homozygous OE-NPY$^{D\beta H}$ mice feeding on chow (Unpublished results). The changes are more severe in male than female OE-NPY$^{D\beta H}$ mice. In line with the lipid storage in tissues, the serum levels of free fatty acids and triglycerides are decreased (Vähätalo et al., 2015b). In contrast, serum total cholesterol levels are increased in older OE-NPY$^{D\beta H}$ mice in both sexes (Unpublished results).

Impaired glucose tolerance and insulin resistance develop subsequent to obesity. In heterozygous OE-NPY$^{D\beta H}$ male mice, impaired glucose tolerance is evident at the age of 6 months and accompanied by an increase in fasting insulin levels (Ruohonen et al., 2008). Heterozygote females do not differ from WT controls unless exposed to Western type diet (Ruohonen et al., 2012). Increased fasting blood glucose levels indicating a high risk for type 2 diabetes are evident only in the homozygous model. Fasting blood glucose is increased in homozygous OE-NPY$^{D\beta H}$ male starting from the age 3 months, but not in female mice (Vähätalo et al., 2015b). Glucose tolerance test points out impaired glucose tolerance in both sexes on chow diet. Insulin tolerance test shows that both sexes have insulin resistance, but significant hyperinsulinemia develops only in OE-NPY$^{D\beta H}$ male mice. The phenotypes of male and female OE-NPY$^{D\beta H}$ mice are summarized in Fig. 1.

3.1. Mechanisms leading to obesity

The postulated mechanisms of noradrenergic NPY leading to obesity in heterozygous and homozygous OE-NPY$^{D\beta H}$ mice are presented in Fig. 2. Noradrenergic NPY can induce obesity via central and/or peripheral mechanisms. Central noradrenergic pathways expressing NPY have been linked to control of vigilance and arousal, and the specific neuronal population from NTS to the paraventricular nucleus in the hypothalamus to hypoglycemia-induced feeding (Li et al., 2009; Heilig, 2004; Sahu et al., 1988; Steinman et al., 1994). Interestingly, obesity in OE-NPY$^{D\beta H}$ mice is not caused by changes in energy intake or expenditure. No differences between genotypes are detected in total or diurnal patterns of feeding of chow or Western diet (Vähätalo et al., 2015b; Ruohonen et al., 2008, 2012). Physical activity over 24 h is mostly similar between the genotypes, except for a small decline in physical activity during the light hours of the day (Vähätalo et al., 2015b). However, this is not reflected to the total energy expenditure measured with indirect calorimetry.

In contrast, when the mice are challenged with an overnight fast, OE-NPY$^{D\beta H}$ mice show increased refeeding (Vähätalo et al., 2015b). The responses to fasting, i.e. increase in serum ghrelin and hypothalamic *Npy* and agouti-related peptide (*Agrp*) mRNA expression, and no change in the hypothalamic pro-opiomelanocorthin (*Pomc*) or brainstem *Npy* expression, are similar between the genotypes. Thus, the increase in refeeding is likely driven by a higher overall expression of NPY.

Peripherally, NPY is acting on several tissues in a way that could promote obesity. In terms of energy intake, the effects of NPY on regulation of gastric motility (Fujimiya and Inui, 2000) and intestinal absorption (Cox, 2007) could play a role. However, no differences in fecal output or fecal lipid content suggest that the development of obesity cannot be explained by differences in nutrient absorption (Vähätalo et al., 2015b).

In WAT, both adipogenic and antilipolytic actions of NPY have previously been reported (Kuo et al., 2008; Valet et al., 1990). Fitting with the adipogenic effect, the significantly smaller size of adipocytes yet