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Chronic Stress, Combined with a High-Fat/High-Sugar Diet, Shifts Sympathetic Signaling toward Neuropeptide Y and Leads to Obesity and the Metabolic Syndrome

Lydia E. Kuo^a, Magdalena Czarnecka^a, Joanna B. Kitlinska^a, Jason U. Tilan^a, Richard Kvetňanský^b, and Zofia Zukowska^a

^aDepartment of Physiology and Biophysics, Georgetown University Medical Center, Washington, DC, USA

^bInstitute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovakia

Abstract

In response to stress, some people lose while others gain weight. This is believed to be due to either increased β -adrenergic activation, the body's main fat-burning mechanism, or increased intake of sugar- and fat-rich "comfort foods." A high-fat, high-sugar (HFS) diet alone, however, cannot account for the epidemic of obesity, and chronic stress alone tends to lower adiposity in mice. Here we discuss how chronic stress, when combined with an HFS diet, leads to abdominal obesity by releasing a sympathetic neurotransmitter, neuropeptide Y (NPY), directly into the adipose tissue. *In vitro*, when "stressed" with dexamethasone, sympathetic neurons shift toward expressing more NPY, which stimulates endothelial cell (angiogenesis) and preadipocyte proliferation, differentiation, and lipid-filling (adipogenesis) by activating the same NPY-Y2 receptors (Y2Rs). *In vivo*, chronic stress, consisting of cold water or aggression in HFS-fed mice, stimulates the release of NPY and the expression of Y2Rs in visceral fat, increasing its growth by 50% in 2 weeks. After 3 months, this results in metabolic syndrome-like symptoms with abdominal obesity, inflammation, hyperlipidemia, hyperinsulinemia, glucose intolerance, hepatic steatosis, and hypertension. Remarkably, local intra-fat Y2R inhibition pharmacologically or via adenoviral Y2R knock-down reverses or prevents fat accumulation and metabolic complications. These studies demonstrated for the first time that chronic stress, via the NPY-Y2R pathway, amplifies and accelerates diet-induced obesity and the metabolic syndrome. Our findings also suggest the use of local administration of Y2R antagonists for treatment of obesity and NPY-Y2 agonists for fat augmentation in other clinical applications.

Keywords

neuropeptide Y; obesity; stress; Y2 receptors; sympathetic system; adipogenesis; angiogenesis; metabolic syndrome

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Address for correspondence: Zofia Zukowska, M.D., Ph.D., Department of Physiology and Biophysics, Georgetown University Medical Center, 3900 Reservoir Road NW, Washington, DC 20057. Voice: +202-687-1034. zzukow01@georgetown.edu.

Conflicts of Interest

The authors declare patent application 11/921,594 filed on 06/06/05.

Introduction

Chronic stress, like aging, is ubiquitous and an accepted part of life. In response to stress, some people lose while others gain weight, which is thought to be attributable to either increased β -adrenergic activation, the body's main fat-burning mechanism or increased intake of sugar- and fat-rich comfort foods.¹ A high-fat, high-sugar (HFS) diet alone, however, cannot account for the current epidemic of obesity,² and chronic stress alone tends to lower adiposity in mice.³ Recently we showed that chronic stress combined with a high-fat, high-sugar diet leads to abdominal obesity by releasing a sympathetic neurotransmitter, neuropeptide Y (NPY), directly into the adipose tissue.⁴ *In vitro*, when "stressed" with dexamethasone, sympathetic neurons shift toward expressing more NPY, which stimulates endothelial cell (angiogenesis) and preadipocyte proliferation, differentiation, and lipid-filling (adipogenesis) by activating the same NPY-Y2 receptors (Y2Rs).⁴

Many determinants, such as genetic pre-disposition, coping mechanisms, and environmental factors, make some individuals more susceptible to morbidity associated with stress. With an increasing prevalence of obesity, recent studies have shown a correlation between stress and the metabolic syndrome.⁵ However, it is perplexing that not all individuals who are stressed gain weight. Some people lose weight during stress, mainly via a β -adrenergic-mediated lipolytic pathway.⁶ Other individuals, those who cope by consuming "comfort foods" rich in fat and sugar, tend to gain weight.¹ Of interest, this weight gain is disproportionate to the number of calories consumed.² This may be a protective mechanism by which, because of perceived stress signals, our bodies crave and store fat.

It is thought that an overactive hypothalamic-pituitary-adrenal axis leading to elevated cortisol levels, as seen in Cushing's syndrome, is to blame for the increased incidence of abdominal obesity.⁷ Although cortisol can lead to adiposity and is secreted during stress, obese individuals were found to be desensitized to glucocorticoids and did not generally exhibit hypercortisolemia.¹ Interestingly, sympathetic activity, which is the body's major stress-activated weight-wasting mechanism via activation of lipolytic β -adrenergic receptors in adipose tissue, appears to be *increased* in human obesity,⁸ suggesting that other factor(s) may be responsible for weight gain.

NPY is an adrenergic cotransmitter and a major stress mediator,⁹ preferentially released from the sympathetic nerves by intense and prolonged stressors. The peptide exerts pleiotropic activities, either synergistic with norepinephrine (NE) (such as vasoconstriction),¹⁰ or antagonistic (such as cardio-depression),¹⁰ or unique to the peptide (such as its centrally mediated ability to stimulate food intake).¹¹ Unlike NE, NPY is a potent growth factor, stimulating cell proliferation and differentiation in a cell- and receptor (R)-specific manner. Via NPY-Y1 receptors (Y1Rs), it is vaso- and neuroproliferative, and modulates immune functions, whereas via Y2Rs, it is angiogenic. The role of NPY-Y5 receptors (Y5Rs) in NPY's actions appears to be that of an auxiliary receptor, the most known of which is its orexigenic activity.¹² We have discovered many of these unique actions of NPY and have established its major role in stress.¹³ Others¹⁴ have documented that increased activity of the NPY-R system plays a role in many forms of experimental obesity. Here we review NPY's peripheral actions in obesity as a stress-inducible adipose tissue growth factor.

What Stressors Favor Adipogenesis?

Our first observation was that not all mice gain weight when stressed. Weight gain also varied depending on background strain, type of diet, and stress. In 2 weeks, wild-type C57BL/6J and 129X1/SvJ mice (strains known to be predisposed to weight gain) did not gain a significant amount of weight on the standard chow rodent diet and tended to gain

weight when given an HFS diet. Their response, in terms of weight gain, also varied depending on what stressors they were exposed to. Stressors, such as restraint, increased plasma NE levels and decreased appetite, thus causing weight loss.¹⁵ Stressors, such as cold-water avoidance, appeared too mild, and the mice adapted to the cold water within a few days, resulting in no change in weight as well. Intense cold (1 h in 0.5 cm of ice-cold water daily) and aggressor (10 min daily with an aggressive alpha-mouse) stressors produced a sustained level of stress to which mice did not appear to habituate, and these stressors increased plasma NPY levels. However, even these stressors did not cause any significant weight gain unless mice were fed an HFS diet. Their reaction can be compared to the condition seen in society today when maladaptation to stress is paired with an HFS diet. Mice fed just an HFS diet and not stressed gained weight about 50% less than those that were additionally stressed.

Changes in Stress Hormones during Chronic Stress and an HFS Diet

The effects of stress on adipose tissue were quite complex. While HFS diet-fed mice had elevated plasma corticosterone, those levels were decreased in mice additionally exposed to stress. However, its converting enzyme, 11 β -hydroxysteroid dehydrogenase (11 β HSD-1), was upregulated during stress, possibly contributing to local intra-fat generation of active glucocorticoids. We also found no correlation between cortisol levels in the fat or plasma and increasing fat mass due to HFS and stress. Although glucocorticoids alone could not stimulate lipid-filling,⁴ they appeared to have primed adipose tissue for the NPY effects by increasing peptide expression in sympathetic nerves. Dexamethasone-treated sympatho-neuronal cells in culture doubled the expression of NPY mRNA after 24 h of treatment, whereas NE-synthesizing enzyme tyrosine hydroxylase mRNA remained the same.⁴ Others have also shown upregulation of NPY expression with glucocorticoids, such as dexamethasone, in various cell types, including neuronal cells and pancreatic islets.^{16–18} Cells in pancreatic islets “switched” from insulin-producing to NPY-producing cells.¹⁷

Similarly, chronically stressed mice did not have elevated plasma and fat NE or epinephrine levels. Of the three stress hormones, only plasma and adipose tissue NPY levels were elevated and were associated with visceral obesity in stressed mice given an HFS diet. Our findings of sympathetic nerve–adipocyte cross-talk were in agreement with studies by Turtzo *et al.*,¹⁴ who showed that co-culture of rat sympathetic neurons with adipocytes raises neuronal expression of NPY, which in turn inhibits the ability of NE to stimulate β -adrenergic lipolysis. Thus, a stress- and glucocorticoid-dependent switch toward over-production of NPY over that of NE may result in antilipolytic and adipogenic effects, facilitating obesity when an HFS diet is given.

Local Effects in White Adipose Tissue

Locally, expression of NPY and its Y2Rs was elevated in the subcutaneous abdominal white adipose tissue (WAT), but not in other fat depots of stressed mice fed the HFS diet. In addition to having more visceral fat volume (quantified by MRI), the mice showed a change in morphology of the subcutaneous WAT, which became multi-locular, enriched in smaller adipocytes, and had a higher expression of brown adipose tissue (BAT)-specific uncoupling protein 1. This was also associated with increased fat vascularization (CD31⁺) and inflammatory cell infiltration (CD68⁺ cells).

Fat is a highly plastic tissue that retains its capacity for remodeling throughout life. This is due to the ability of existing adipocytes to grow in size by filling up with lipids, but also because of the presence of preadipocytes, which, when given proper growth factors, can proliferate and differentiate into mature fat cells. We confirmed *in vitro* that all of these processes are Y2R-dependent. The Y2R involvement with the (pre)adipocytes was

paralleled in the vessels that supplied the adipose tissue. NPY exerts potent angiogenic effects in multiple tissues,^{19–21} including fat.⁴ Inhibition of Y2Rs with a specific Y2R antagonist (BIIE 0426; Tocris Bioscience, Ellisville, MO) or receptor deficiency due to adenoviral transfer of Cre-recombinase into Y2-floxed mice, resulted in apoptosis of endothelial and fat cells, inhibition of proliferation of new preadipocytes, and immune cell infiltration. Overall, this caused a 40% reduction in vascularization and fat deposition in the visceral fat within 2 weeks after local treatment of that depot.

We have also tested the converse and applied exogenous NPY in a slow-release pellet under the skin of a lean or obese mouse, or in nude mice with a human fat xenograft. NPY markedly increased murine fat growth and sustained survival and stimulated vascularization of the human graft, which otherwise, without NPY, became almost fully absorbed within 3 months.

Proposed Mechanism

On the basis of our results, we propose the following scheme (Fig. 1): Chronic stress, to which animals or humans cannot easily adapt—such as aggression or cold—when combined with an HFS “comfort food” diet, stimulates sympathetic nerves to upregulate NPY expression, without a proportional increase in NE. Stress and an HFS diet also increases glucocorticoids levels, primarily in the visceral fat, which in turn further upregulate expression of NPY and its Y2Rs in fat tissues. Stimulation of these receptors on endothelial and fat cells stimulates angiogenesis and adipogenesis and leads to fat growth, preferentially in the abdominal area. With time, this leads to inflammation of the fat tissue, hypervascularization, and hormonal changes, such as hyperinsulinemia and hyperlipidemia. After 3 months of stress, HFS-fed mice develop a metabolic-like syndrome. Local intra-fat inhibition of Y2Rs prevents or reverses fat accumulation by inducing apoptosis of endothelial and fat cells, and improves systemic metabolic consequences.

Conclusions

The discovery of the stress-activated NPY-Y2R-mediated pathway opens new avenues for treatment of abdominal obesity with local administration of Y2R antagonists. It also presents a novel way of using NPY-Y2R agonists for increasing fat survival for other clinical applications, such as fat augmentation or grafting in reconstructive surgery. Interestingly, our findings are corroborated by studies in Northern European populations.²² A Y2R-silent mutation in a Swedish population²³ is associated with resistance to obesity, while a gain-of-function polymorphism in the NPY gene appears to predispose the individual to hyperlipidemia, atherosclerosis, and severe complications of type II diabetes. Thus, an overactive NPY-Y2R system may be a factor predisposing an individual to the metabolic syndrome, whereas silencing of Y2Rs may protect against it.

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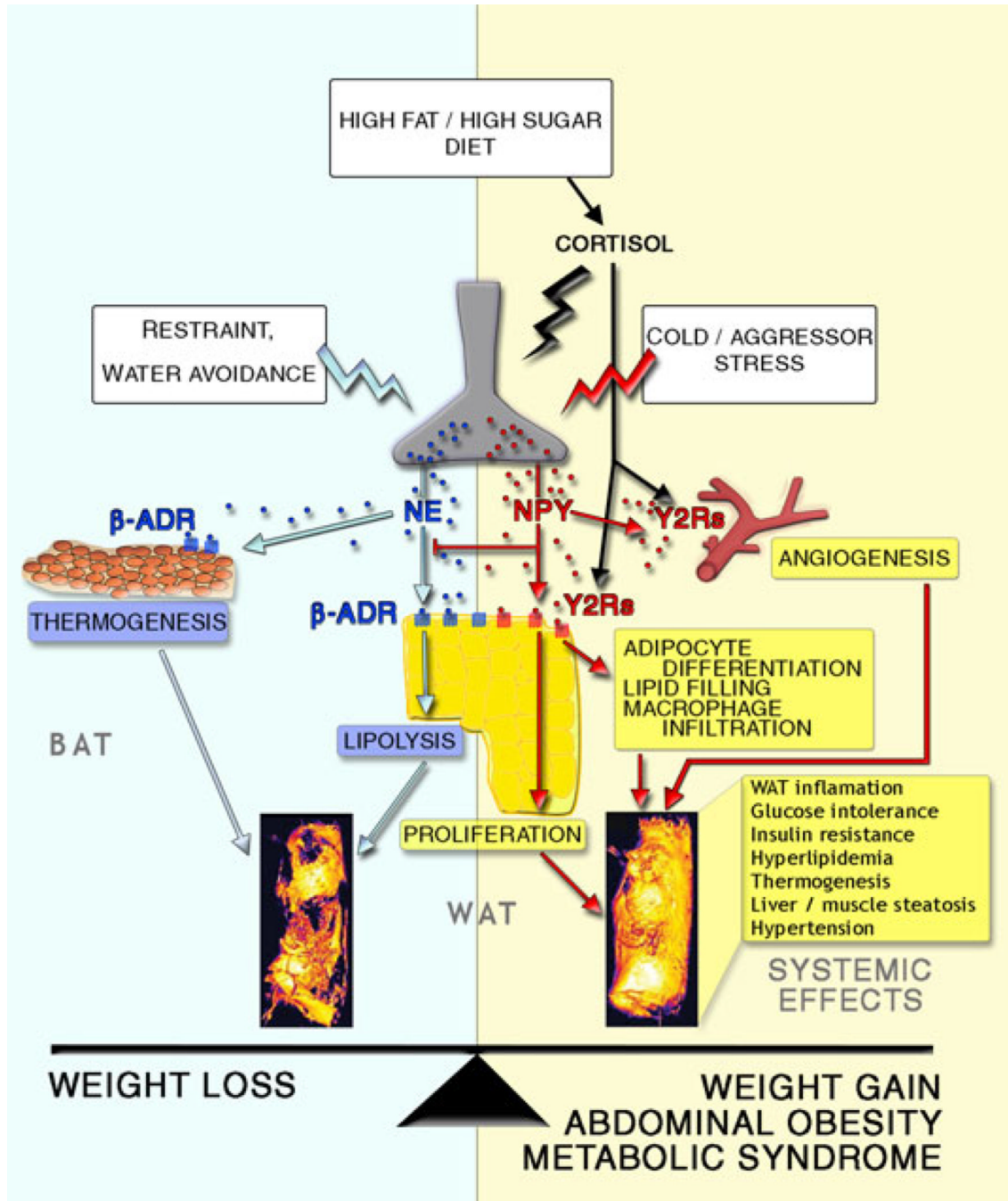


Figure 1. Role of NPY and its Y2Rs in stress-induced obesity. Stress of exposure to cold or aggression activates sympathetic nerves and preferentially releases NPY over NE from the sympathetic nerves in mice. Stress of restraint or water avoidance preferentially releases NE, without significant increase in NPY; this results in increased β -adrenergic thermogenesis in brown adipose tissue (BAT) and lipolysis in white adipose tissue (WAT), leading to weight loss in both cases. Feeding the cold- or aggressor-stressed mice an HFS diet increases the adipose tissue levels of cortisol, which in turn, further upregulates expression of NPY in the sympathetic nerves and NPY-Y2Rs in the adipocytes and endothelial cells. Activation of these receptors stimulates proliferation, differentiation, and lipid filling of adipocytes,

angiogenesis, and macrophage infiltration, and leads to abdominal fat growth. After 3 months of that stress and an HFS diet, mice develop gross abdominal obesity and metabolic-like syndrome.