Symposium

Neuropeptide Y is a mediator of chronic vascular and metabolic maladaptations to stress and hypernutrition

Ken Abe, Lydia Kuo and Zofia Zukowska

Department of Physiology and Biophysics, Georgetown University Medical Center, 3900 Reservoir Rd NW, Washington, DC 20057, USA
Corresponding author: Zofia Zukowska. Email: zzukow01@georgetown.edu

Abstract

Neuropeptide Y (NPY) is a central neuromodulator and peripheral sympathetic neurotransmitter that also has important regulatory roles in cardiovascular, neuroendocrine, immune and metabolic functions during stress. Focusing on the peripheral actions of the peptide in rodent models, we summarize recent studies from our laboratory demonstrating that stress-induced release of NPY mediates accelerated atherosclerosis/restenosis, obesity and metabolic-like syndrome, particularly when combined with a high fat, high sugar diet. In this review, we propose mechanisms of NPY's actions, its receptors and cellular substrates that increase the risk for cardiovascular and metabolic diseases when chronic stress is associated with pre-existing vascular injury and/or states of hypernutrition.

Keywords: atherosclerosis, cardiovascular disease, metabolic syndrome, neuropeptide Y, obesity, stress

Experimental Biology and Medicine 2010; **235**: 1179–1184. DOI: 10.1258/ebm.2010.009136

Introduction

Obesity, cardiovascular and metabolic diseases are primary causes of mortality in the Western world. In the United States, approximately 65% of adults are overweight with a body mass index (BMI) greater than 25 kg/m^2. According to the National Health and Nutrition Survey for 1999–2000, 31% of those in the overweight classification are obese with BMIs >30. This overall rise has been followed by a higher incidence of abdominal obesity, a particular type of obesity that has been linked to metabolic syndrome.[1] This condition comprises a variety of risk factors for cardiovascular disease with symptoms that include hypertension, hyperlipidemia, glucose intolerance, insulin resistance and low-grade inflammation. While a diet rich in fat and sugars combined with a sedentary lifestyle are key factors contributing to the obesity epidemic leading to metabolic syndrome, genetic and environmental factors are also likely to play a causative role. Among these genetic and environmental factors, stress has been anecdotally linked to obesity and is generally acknowledged to be a risk factor for heart disease, but basic research data in support of these claims have been largely missing and descriptive mechanisms still remain unknown.

An emerging candidate relating stress-induced obesity and perhaps metabolic syndrome is neuropeptide Y (NPY). NPY is an abundant neuromodulator in the brain and has long been implicated as being one of the body's most potent orexigenic factors, although the importance of this mechanism continues to be under scrutiny. NPY is also a neurotransmitter released in the periphery from the sympathetic nerves and adrenal medulla during stress.[2–4] Its release has been linked to a variety of stress-induced changes in the cardiovascular, immune and endocrine systems, whose actions are dependent on the activation of multiple G-protein-coupled receptors, Y1–Y5. Our studies conducted over the course of the last decade has led us to propose that NPY is a master stress hormone, coordinating cardiovascular, neuroendocrine, immune and metabolic adaptations to stress, which once may have served an evolutionary adaptive role but has since become maladaptive in modern human societies. In this review, we will present some of our observations that support this notion.

NPY is a mediator of proatherosclerotic effects of chronic stress

Since its discovery in 1982,[5] NPY has been known for its direct effects on vasoconstriction, which was found to be mediated through the Y1 receptor (R). Additionally, this peptide contributes to vascular tone by potentiating the actions of other vasoconstrictors, primarily norepinephrine (NE) and epinephrine (Epi).[6] Subsequently, our[7–9] and other[10,11] studies have determined that NPY also stimulates

vascular smooth muscle cell (VSMC) proliferation and hypertrophy via the same Y1 receptor. *In vivo*, this activity leads to NPY's potent effects to stimulate neointima formation after angioplasty.[12,13] In a rat model of carotid artery balloon angioplasty-induced injury, a placement of a slow-releasing NPY pellet (1–10 μg/14 d) led to the formation of an occlusive atherosclerotic-like lesion containing not only neointima, but also lipids, macrophages, matrix, thrombus and neovessels. This occurred in an animal model (rat) devoid of metabolic abnormalities and in the absence of an atherosclerosis-promoting diet. Most remarkably, the NPY-induced vascular occlusive lesion was significantly attenuated by a continuous intravenous infusion of an Y1R antagonist. An antagonist to another NPY receptor, Y5, was also effective, although to a lesser degree, and the combined Y1 and Y5R inhibition did not exert any synergistic effects.[13]

Physiologically, NPY is released during stress, along with NE, but proportions of these two transmitters vary depending on the type of stress.[14] Unlike NE which is secreted during the mildest acute stress and is considered a good marker of a 'stressed' animal, the release of NPY requires a more prolonged and/or intense stimulation of sympathetic nerves. Intense stressors such as the placement of rodents in shallow ice-cold water (1 cm) for 1 h/d or a 10-min exposure to an aggressive mouse (but not a mild stress of restraint) release NPY from the sympathetic nerves and elevate plasma NPY levels.[4,14–17]

In rats and some murine strains, there is an additional source of circulating NPY found in platelets,[18] and is derived from its synthesis during the megakaryocyte stage of development.[19,20] The surgical stress of angioplasty alone was found to be sufficient to increase plasma and platelet NPY levels, and these effects were further augmented by cold stress in rats.[13] Two weeks of daily exposure to cold stress fully mimicked the effects of the NPY pellet and resulted in a total occlusion of rat carotid artery. Similar to the exogenously added NPY, the occlusive lesion was composed of infiltrated macrophages, thrombus, matrix, lipid deposition and neovessels. Furthermore, these effects were attenuated by the Y1R antagonist,[12] indicating NPY's activity along similar pathways.

The important question arising from these studies was – how could a single peptide via its one or two receptors mediate such a complex vascular, immune and metabolic response to injury, leading to an atherosclerotic-like lesion? One possibility would be that NPY acts as a trigger of a cascade of events, or that it is a mediator of a final common pathway used by a variety of growth and metabolic factors. This latter notion, although attractive, is unlikely as these functions are usually governed by multiple intracellular signaling molecules, and given the fact that genetic deletions of NPY are not lethal in the mouse. However, the possibility of a role for NPY in triggering these events is more likely considering the fact that it is released from the nerves at the site of injury, and thus may be well positioned to activate responses from multiple cell types surrounding these nerve terminals.

Several observations from recent studies have supported this latter notion. First, NPY is found to exert proinflammatory actions on immune cells by stimulating monocyte migration,[21–24] leukocyte phagocytosis and release of inflammatory mediators. At least some of these effects appear to involve Y1Rs on antigen-presenting cells, such as macrophages, as shown by Wheway *et al.*[25] In our studies, both stress and NPY similarly stimulated macrophage infiltration and upregulated Y1R in these cells. Second, *in vitro*, NPY is a potent mitogen for VSMCs via Y1 and to a lesser degree, Y5Rs.[7] *In vivo*, cells within the neointima are highly proliferative while having an upregulation of Y1R expression after stress or exogenous NPY treatment.[12] Third, NPY is also potently angiogenic for endothelial cells (EC) that primarily involve Y2R, with an upregulation of both Y1 and Y5Rs in this process.[26–31] Supporting the angiogenic action of NPY in stress- and NPY-induced postangioplasty atherosclerotic lesions, there was a significant increase in neovascularization of the neointima and upregulation of all NPY receptors within these microvessels.[12,13] Finally, stress was found to elevate circulating NPY levels in platelets.[13] This physiological significance of having an elevation of platelet NPY content and their receptors are currently being studied. However, there is already some data supporting the role of NPY at the level of the bone marrow in platelets and with immune cells,[20,32–35] which may in turn lead to increased thrombosis and atherosclerotic vascular remodeling.

How the direct actions of NPY and stress contribute to postangioplasty restenosis or atherosclerosis in humans still remain an active area of study. Several promising studies support this link, including a recent study implicating NPY and the Y1 receptor in arterial remodeling using $ApoE^{-/-}$ mice.[36] This study is further supported in a previous study in humans, in which a leucine7 to proline7 polymorphism in prepro-NPY was found to be associated with increased blood pressure, neointimal thickness and total serum cholesterol.[37] Lastly, in a recent study from Ruohonen *et al.*,[38] we were able to demonstrate that overexpression of NPY in mice was found to increase postangioplasty arterial remodeling. Combined, these animal and human studies suggest that NPY is likely to be a risk factor for accelerated atherosclerosis/restenosis, and that NPY could serve as both a novel stress and proatherosclerotic marker and a mediator of these effects.

NPY is a mediator of pro-obesity and metabolic effects of chronic stress

Stress has also been linked to the pathogenesis of obesity. However, their relationship remains unclear, as some people lose weight when stressed, while others gain. The cause has been thought to be due to central regulation and differences in the perception of stress or differential coping patterns, i.e. alterations in food intake, sympathetic nerve activity and/or hypothalamic-pituitary-adrenocortical function.[39] Nonetheless, the question still remains: is the stress–body relationship only in the brain?

Human obesity is often associated with increased sympathetic activity.[40] However, sympathetic release of NE and the activation of its β-adrenergic receptor promote

weight loss by stimulating lipolysis in white adipose tissue (WAT)[41] and thermogenesis in brown adipose tissue (BAT).[42] Hence, in obesity, β-adrenergic activity may be downregulated and/or offset by more powerful mechanisms that promote weight gain. We hypothesized that NPY could be a critical factor by acting on one or more of the following mechanisms through the stimulation of (1) angiogenesis (indirectly increasing fat tissue growth by augmenting nutrient supply) and (2) new adipocyte proliferation and differentiation and lipid storage, and by the (3) inhibition of β-adrenergic lipolysis. Since the latter has been well established as reviewed by Langin,[41] we focused on addressing the first two possibilities.[17]

We used the same stress paradigm in mice as the one which previously increased circulating NPY in rodents by standing in shallow cold water (0.5 cm) one hour daily or exposure to an aggressive alpha-mouse for 10 min. Both stressors chronically elevated not only plasma corticosterone, but also plasma and/or platelet NPY levels; however, by itself this did not affect adiposity and body weight of mice within two weeks of observation. However, when either stressor was combined with a high fat, high sugar (HFS) diet, mice increased their fat deposition by 40–50% in two weeks, as compared with unstressed mice on the same diet. The largest fat accumulation was in the abdominal area (as measured by magnetic resonance imaging [MRI] for combined subcutaneous and intra-abdominal fat depots) and was not accompanied by any significant changes in food intake, fecal output or core body temperature. Stress-induced increases in abdominal adiposity was accompanied by increased vascularization (CD31 immunostaining) and cell proliferation (Ki67 immunostaining) and macrophage (CD68 immunostaining) infiltration within the abdominal fat, and also impaired glucose tolerance as compared with unstressed HFS diet-fed mice.

Interestingly, while plasma levels of the two major stress mediators, corticosterone and NE, normalized in the chronically stressed, HFS diet-fed mice, circulating NPY levels remained high or even increased. In addition, there was a marked upregulation of 11 β-HSD-1 and increased corticosterone levels in the abdominal fat, as well as two- to seven-fold increase in the expression of NPY, its Y2R and dipeptidyl peptidase IV (DPPIV), which converts NPY to a shorter Y2/Y5R-selective form of the peptide. We subsequently found that such an increase in glucocorticoid signaling in the abdominal WAT was likely an additional factor that augmented stress/HFS-induced upregulation of NPY expression in sympathetic neurons (based on *in vitro* studies using neuroblastoma cells as a model) and Y2R in preadipocytes (PA) (using 3T3-L1 cells as a model). No such stimulation was observed for the adrenergic system leading to a predominance of NPY-mediated signaling within the abdominal fat depot.

The functional significance of augmented NPY activity within the WAT was first determined *in vitro* using EC and PA cultured alone or with sympathetic neuronal cells (neuroblastoma). In both cell types, NPY, either synthetic or released from the neuronal cells, stimulated cell proliferation which was blocked/inhibited by an Y2R antagonist. The co-culture with neuroblastoma cells also upregulated Y2R in EC and PA. Additionally, in PA that were primed for differentiation, NPY (0.1–10 nmol/L) had a direct stimulatory effect on adipogenesis in a manner similar to insulin (lipid accumulation and leptin and resitin secretion). These studies indicated that NPY is a potent angiogenic and adipogenic factor by activating the same receptor, Y2R.

To confirm this *in vivo*, an NPY pellet (1 μg/14 d) was placed subcutaneously into the abdominal fat of several types of mice: normal lean controls on C57/BL6 and Sv129/J backgrounds, and ob/ob mice. After two weeks, NPY induced a 40–50% increase in fat deposition in a 2 cm area from the pellet (by fat weight and MRI), with increased vascularization and proliferation of new small adipocytes, indicating angiogenesis and adipogenesis.

To determine the relevance of these findings to humans, human fat from liposuction was placed under the skin of athymic mice, and treated with either a placebo or an NPY pellet. After three months, placebo-treated fat depot became almost completely resorbed, while NPY-treated fat persisted and became well vascularized. The same Y2R (as well as NPY itself) was expressed in capillaries and adipocytes of human fat, and was markedly upregulated following NPY treatment. These observations confirmed that both mice and humans have a similar NPY-Y2R system, which functions to stimulate angiogenesis and adipogenesis, and thus may be involved in the development of obesity in both species.

The role of NPY-Y2R system in stress-induced obesity was then studied using either a local intrafat administration of Y2R antagonist (daily injections subcutaneously or a slow-release pellet), a germline Y2R deletion or in mice where Y2R expression was 80% reduced by an injection of an adenoviral vector with Cre-recombinase into $Y2^{lox/lox}$ mice. In all of these conditions, there was a 40–50% decrease in the abdominal fat depots within two weeks through inhibition of stress-induced adipogenesis. This was accompanied by increased apoptosis (terminal deoxynucleotidyl transferase dUTP nick end labeling staining) and decreased proliferation of ECs and adipocytes, and a reduction in CD68-positive macrophages within the abdominal fat depot.

After three months of stress and HFS diet, mice became grossly obese, almost twice as much as unstressed mice on the same diet, and developed a metabolic-like syndrome with abdominal obesity, elevated blood pressure (measured by telemetry), glucose intolerance, insulin resistance, hyperlipidemia, fat inflammation, as well as liver and skeletal muscle steatosis. Most of these metabolic changes were markedly reduced by local intrafat administration of an Y2R antagonist or in $Y2^{-/-}$ mice. Thus, we concluded that stress augments fat growth, development of abdominal obesity and the onset of metabolic syndrome by activating the NPY-Y2R system preferentially within the abdominal fat. The adipogenic activity of NPY is both indirect (by stimulating fat vascularization) as well as direct (by its ability to stimulate formation of new fat cells, promoting differentiation of PA and lipid storage in adipocytes). Remarkably, a single receptor antagonist against the Y2R appears to not only prevent stress-induced obesity, but

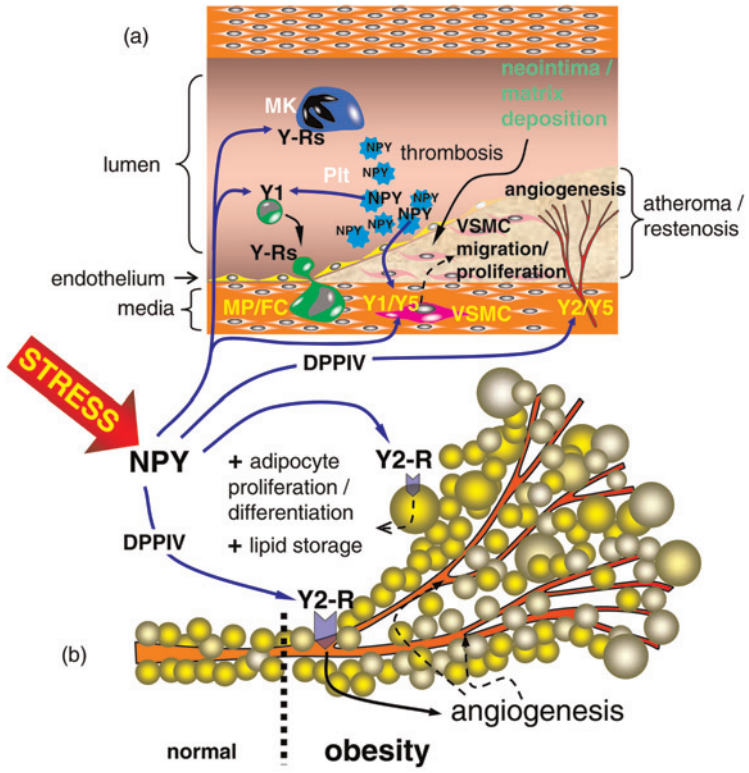

Figure 1 Stress-induced neuropeptide Y (NPY) effects on vascular occlusion remodeling and obesity. (a) Stress-released NPY directly stimulates neointimal formation through modulation of monocytes (MC) and the promotion of macrophage/foam cells (MP/FC) after endothelial injury, while also directly stimulating vascular smooth muscle cells (VSMC) proliferation and migration through innervation. Additionally, stress, possibly through NPY, stimulates increased megakaryocyte (MK) turnover via Y5 activation resulting in both increased platelet (Plt) synthesis and platelet content of NPY. Platelet NPY serves as an additional source of NPY for MC and VSMC. Cleavage of 1–36 NPY to the proangiogenic 3–36 NPY via dipeptidyl peptidase IV acts on Y2/Y5 Rs to promote angiogenesis, thus providing increased nutrient supply to the occlusive vascular lesion. (b) NPY induction after stress coordinates the combined direct effects of adipocyte proliferation/differentiation and lipid storage, while also promoting the indirect role of angiogenesis, which also increases nutrient supply to increase adiposity, and thus obesity

also reverse fat accumulation in either normal lean or genetically obese mice.

Interestingly, in humans, a silencing mutation in the Y2R gene appears to also be associated with resistance to obesity in Swedes.[43] Thus, we propose a novel pathway by which fat tissue remodels itself in response to sympathetic nerve activation during stress and overfeeding. Our findings provide a novel avenue for therapy of abdominal obesity and metabolic syndrome with locally acting Y2R antagonists, as well as the potential use of NPY-like drugs for fat grafting in reconstructive plastic surgery.

Conclusions

The regulatory mechanisms governing metabolism and its dysregulation are complex. In an attempt to address these problems, we have grown increasingly aware that a cure for one ailment can lead to others. Such could be the case of a promising drug (a DPPIV/C26 inhibitor) to treat diabetes[44] that could inadvertently elevate blood pressure, resulting in increased mortality due to stroke and ischemic heart disease. In this example, inhibition of DPPIV prevents endogenous cleavage of intact NPY_{1-36}, which can then activate vasoconstrictive Y1Rs.[45,46] The increased urgency, therefore, is to understand seemingly distinct entities in metabolic diseases from a more global perspective in order to gain insight into previously unknown relationships.

Can our studies on the peripheral effects of NPY help us to gain this broader understanding of metabolic diseases? It appears that as we have uncovered more about the action of NPY that the answer is yes. NPY is well poised to serve as a model for other known regulatory factors in metabolic processes, owing to its pleiotropic effects to multiple cellular substrates and multiple receptor subtypes (Y1–Y6, and more recently, Y7). These effects are further complicated by the receptors' dependence on location, expression levels and other factors (e.g. glucocorticoids or catecholamines). As such, NPY is emerging as a prime candidate as a master regulatory peptide of cardiovascular, immune and metabolic functions.

These actions of NPY may have been adaptive in the animal kingdom, particularly in colder climates, in periods of sparse food availability and also perhaps during hibernation.[47,48] Similarly in humans, a hyperactive NPY system could have offered an evolutionary protection by increasing cardiovascular performance and ability to build energy stores (fat) in cold environments with poor availability of food. Interestingly, a Leu7Pro polymorphism in the NPY signal peptide gene which apparently increases the amount of NPY released during stress[49] occurs commonly in populations within the colder regions of Northern

Europe, but is absent in warmer areas such as Africa and South Asia.

The adaptive role of NPY, however, appears to change when stress occurs with vascular injury, inflammation and hypernutrition, which have become increasingly frequent in populations of the Western world. When stress is combined with underlying atherosclerosis and vascular injury such as balloon angioplasty or stenting, and/or with a HFS diet, the subsequent increase in NPY levels becomes maladaptive. The recent findings from our group, and more recently from others,[50,51] call to attention the pervasive effects of NPY on endothelial, VSMC, immune, fat and other cells that have been previously overlooked in studies dealing with metabolic regulation (Figure 1). Collectively, these studies provided evidence that NPY is adipogenic and angiogenic for murine,[17] rat[51] and human[50] fat, in particular within the abdominal depot, thus contributing to the development of metabolic syndrome.

In conclusion, we propose that NPY is a stress-induced risk for cardiovascular diseases, abdominal obesity and metabolic syndrome, particularly when stress is combined with hypernutrition. Understanding how NPY and its cognate receptors regulate metabolic and vascular functions may therefore provide gainful insight into future therapies.

Author contributions: KA prepared and wrote this manuscript detailing the effects of NPY on cardiovascular-related diseases. KA also prepared the figure in this review. LK contributed her expertise regarding the role of NPY in obesity, and was responsible for writing and preparing this portion of the review. ZZ was responsible for the final editing and proofing of the manuscript.

ACKNOWLEDGEMENTS

This work was funded by Grant No. 5R01HL067357-09, 'NPY and Angiogenesis and Adipose Tissue'.

Conflict of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES

1 Ritchie SA, Connell JM. The link between abdominal obesity, metabolic syndrome and cardiovascular disease. *Nutr Metab Cardiovasc Dis* 2007;**17**:319–26
2 Morris M, Kapoor V, Chalmers J. Plasma neuropeptide Y concentration is increased after hemorrhage in conscious rats: relative contributions of sympathetic nerves and the adrenal medulla. *J Cardiovasc Pharmacol* 1987;**9**:541–5
3 Zukowska-Grojec Z, Konarska M, McCarty R. Differential plasma catecholamine and neuropeptide Y responses to acute stress in rats. *Life Sci* 1988;**42**:1615–24
4 Zukowska-Grojec Z, Vaz AC. Role of neuropeptide Y (NPY) in cardiovascular responses to stress. *Synapse* 1988;**2**:293–8
5 Tatemoto K. Neuropeptide Y: complete amino acid sequence of the brain peptide. *Proc Natl Acad Sci USA* 1982;**79**:5485–9
6 Fallgren B, Arlock P, Edvinsson L. Neuropeptide Y potentiates noradrenaline-evoked vasoconstriction by an intracellular calcium-dependent mechanism. *J Auton Nerv Syst* 1993;**44**:151–9
7 Pons J, Kitlinska J, Ji H, Lee EW, Zukowska Z. Mitogenic actions of neuropeptide Y in vascular smooth muscle cells: synergetic interactions with the beta-adrenergic system. *Can J Physiol Pharmacol* 2003;**81**:177–85
8 Pons J, Lee EW, Li L, Kitlinska J. Neuropeptide Y: multiple receptors and multiple roles in cardiovascular diseases. *Curr Opin Invest Drugs* 2004;**5**:957–62
9 Zukowska-Grojec Z, Pruszczyk P, Colton C, Yao J, Shen GH, Myers AK, Wahlestedt C. Mitogenic effect of neuropeptide Y in rat vascular smooth muscle cells. *Peptides* 1993;**14**:263–8
10 Erlinge D, Brunkwall J, Edvinsson L. Neuropeptide Y stimulates proliferation of human vascular smooth muscle cells: cooperation with noradrenaline and ATP. *Regul Pept* 1994;**50**:259–65
11 Nie M, Selbie LA. Neuropeptide Y Y1 and Y2 receptor-mediated stimulation of mitogen-activated protein kinase activity. *Regul Pept* 1998;**75–76**:207–13
12 Li L, Jonsson-Rylander AC, Abe K, Zukowska Z. Chronic stress induces rapid occlusion of angioplasty-injured rat carotid artery by activating neuropeptide Y and its Y1 receptors. *Arterioscler Thromb Vasc Biol* 2005;**25**:2075–80
13 Li L, Lee EW, Ji H, Zukowska Z. Neuropeptide Y-induced acceleration of postangioplasty occlusion of rat carotid artery. *Arterioscler Thromb Vasc Biol* 2003;**23**:1204–10
14 Zukowska-Grojec Z. Neuropeptide Y. A novel sympathetic stress hormone and more. *Ann NY Acad Sci* 1995;**771**:219–33
15 Allen JM, Schon F, Yeats JC, Kelly JS, Bloom SR. Effect of reserpine, phenoxybenzamine and cold stress on the neuropeptide Y content of the rat peripheral nervous system. *Neuroscience* 1986;**19**:1251–4
16 Castagne V, Corder R, Gaillard R, Mormede P. Stress-induced changes of circulating neuropeptide Y in the rat: comparison with catecholamines. *Regul Pept* 1987;**19**:55–63
17 Kuo LE, Kitlinska JB, Tilan JU, Li L, Baker SB, Johnson MD, Lee EW, Burnett MS, Fricke ST, Kvetnansky R, Herzog H, Zukowska Z. Neuropeptide Y acts directly in the periphery on fat tissue and mediates stress-induced obesity and metabolic syndrome. *Nat Med* 2007;**13**:803–11
18 Myers AK, Torres Duarte AP, Zukowska-Grojec Z. Immunoreactive neuropeptide Y (NPY) in plasma and platelets of rat and mouse strains and human volunteers. *Regul Pept* 1993;**47**:239–45
19 Ericsson A, Larhammar D, McIntyre KR, Persson H. A molecular genetic approach to the identification of genes expressed predominantly in the neuroendocrine and immune systems. *Immunol Rev* 1987;**100**:261–77
20 Ericsson A, Schalling M, McIntyre KR, Lundberg JM, Larhammar D, Seroogy K, Hokfelt T, Persson H. Detection of neuropeptide Y and its mRNA in megakaryocytes: enhanced levels in certain autoimmune mice. *Proc Natl Acad Sci USA* 1987;**84**:5585–9
21 Bedoui S, Kawamura N, Straub RH, Pabst R, Yamamura T, von Horsten S. Relevance of neuropeptide Y for the neuroimmune crosstalk. *J Neuroimmunol* 2003;**134**:1–11
22 Bedoui S, Kuhlmann S, Nave H, Drube J, Pabst R, von Horsten S. Differential effects of neuropeptide Y (NPY) on leukocyte subsets in the blood: mobilization of B-1-like B-lymphocytes and activated monocytes. *J Neuroimmunol* 2001;**117**:125–32
23 Straub RH, Mayer M, Kreutz M, Leeb S, Scholmerich J, Falk W. Neurotransmitters of the sympathetic nerve terminal are powerful chemoattractants for monocytes. *J Leukocyte Biol* 2000;**67**:553–8
24 Schwarz H, Villiger PM, von Kempis J, Lotz M. Neuropeptide Y is an inducible gene in the human immune system. *J Neuroimmunol* 1994;**51**:53–61
25 Wheway J, Herzog H, Mackay F. The Y(1) receptor for NPY: a key modulator of the adaptive immune system. *Peptides* 2007;**28**:453–8
26 Ekstrand AJ, Cao R, Bjorndahl M, Nystrom S, Jonsson-Rylander AC, Hassani H, Hallberg B, Nordlander M, Cao Y. Deletion of neuropeptide Y (NPY) 2 receptor in mice results in blockage of NPY-induced angiogenesis and delayed wound healing. *Proc Natl Acad Sci USA* 2003;**100**:6033–8
27 Grant DS, Zukowska Z. Revascularization of ischemic tissues with SIKVAV and neuropeptide Y (NPY). *Adv Exp Med Biol* 2000;**476**:139–54
28 Lee EW, Grant DS, Movafagh S, Zukowska Z. Impaired angiogenesis in neuropeptide Y (NPY)-Y2 receptor knockout mice. *Peptides* 2003;**24**:99–106

29 Lee EW, Michalkiewicz M, Kitlinska J, Kalezic I, Switalska H, Yoo P, Sangkharat A, Ji H, Li L, Michalkiewicz T, Ljubisavljevic M, Johansson H, Grant DS, Zukowska Z. Neuropeptide Y induces ischemic angiogenesis and restores function of ischemic skeletal muscles. *J Clin Invest* 2003;**111**:1853–62

30 Movafagh S, Hobson JP, Spiegel S, Kleinman HK, Zukowska Z. Neuropeptide Y induces migration, proliferation, and tube formation of endothelial cells bimodally via Y1, Y2, and Y5 receptors. *FASEB J* 2006;**20**:1924–6

31 Zukowska-Grojec Z, Karwatowska-Prokopczuk E, Rose W, Rone J, Movafagh S, Ji H, Yeh Y, Chen WT, Kleinman HK, Grouzmann E, Grant DS. Neuropeptide Y: a novel angiogenic factor from the sympathetic nerves and endothelium. *Circ Res* 1998;**83**:187–95

32 Ericsson A, Hemsen A, Lundberg JM, Persson H. Detection of neuropeptide Y-like immunoreactivity and messenger RNA in rat platelets: the effects of vinblastine, reserpine, and dexamethasone on NPY expression in blood cells. *Exp Cell Res* 1991;**192**:604–11

33 Hemsen A, Pernow J, Millberg BI, Lundberg JM. Effects of vinblastine on neuropeptide Y levels in the sympathoadrenal system, bone marrow and thrombocytes of the rat. *Agents Actions* 1991;**34**:429–38

34 Tabarowski Z, Gibson-Berry K, Felten SY. Noradrenergic and peptidergic innervation of the mouse femur bone marrow. *Acta Histochem* 1996;**98**:453–7

35 Bedoui S, von Horsten S, Gebhardt T. A role for neuropeptide Y (NPY) in phagocytosis: implications for innate and adaptive immunity. *Peptides* 2007;**28**:373–6

36 Shah SH, Freedman NJ, Zhang L, Crosslin DR, Stone DH, Haynes C, Johnson J, Nelson S, Wang L, Connelly JJ, Muehlbauer M, Ginsburg GS, Crossman DC, Jones CJ, Vance J, Sketch MH, Granger CB, Newgard CB, Gregory SG, Goldschmidt-Clermont PJ, Kraus WE, Hauser ER. Neuropeptide Y gene polymorphisms confer risk of early-onset atherosclerosis. *PLoS Genet* 2009;**5**:e1000318

37 Karvonen MK, Valkonen VP, Lakka TA, Salonen R, Koulu M, Pesonen U, Tuomainen TP, Kauhanen J, Nyyssonen K, Lakka HM, Uusitupa MI, Salonen JT. Leucine7 to proline7 polymorphism in the preproneuropeptide Y is associated with the progression of carotid atherosclerosis, blood pressure and serum lipids in Finnish men. *Atherosclerosis* 2001;**159**:145–51

38 Ruohonen ST, Abe K, Kero M, Toukola L, Ruohonen S, Roytta M, Koulu M, Pesonen U, Zukowska Z, Savontaus E. Sympathetic nervous system-targeted neuropeptide Y overexpression in mice enhances neointimal formation in response to vascular injury. *Peptides* 2009;**30**:715–20

39 Torres SJ, Nowson CA. Relationship between stress, eating behavior, and obesity. *Nutrition* 2007;**23**:887–94

40 Turtzo LC, Lane MD. Completing the loop: neuron–adipocyte interactions and the control of energy homeostasis. *Horm Metab Res* 2002;**34**:607–15

41 Langin D. Adipose tissue lipolysis as a metabolic pathway to define pharmacological strategies against obesity and the metabolic syndrome. *Pharmacol Res* 2006;**53**:482–91

42 Robidoux J, Martin TL, Collins S. Beta-adrenergic receptors and regulation of energy expenditure: a family affair. *Annu Rev Pharmacol Toxicol* 2004;**44**:297–323

43 Lavebratt C, Alpman A, Persson B, Arner P, Hoffstedt J. Common neuropeptide Y2 receptor gene variant is protective against obesity among Swedish men. *Int J Obes (Lond)* 2006;**30**:453–9

44 Combettes M, Kargar C. Newly approved and promising antidiabetic agents. *Therapie* 2007;**62**:293–310

45 Han S, Chen X, Cox B, Yang CL, Wu YM, Naes L, Westfall T. Role of neuropeptide Y in cold stress-induced hypertension. *Peptides* 1998;**19**:351–8

46 Han S, Yang CL, Chen X, Naes L, Cox BF, Westfall T. Direct evidence for the role of neuropeptide Y in sympathetic nerve stimulation-induced vasoconstriction. *Am J Physiol* 1998;**274**:H290–4

47 Dark J, Pelz KM. NPY Y1 receptor antagonist prevents NPY-induced torpor-like hypothermia in cold-acclimated Siberian hamsters. *Am J Physiol Regul Integr Comp Physiol* 2008;**294**:R236–45

48 Gluck EF, Stephens N, Swoap SJ. Peripheral ghrelin deepens torpor bouts in mice through the arcuate nucleus neuropeptide Y signaling pathway. *Am J Physiol Regul Integr Comp Physiol* 2006;**291**:R1303–9

49 Kallio J, Pesonen U, Karvonen MK, Kojima M, Hosoda H, Kangawa K, Koulu M. Enhanced exercise-induced GH secretion in subjects with Pro7 substitution in the prepro-NPY. *J Clin Endocrinol Metab* 2001;**86**:5348–52

50 Kos K, Harte AL, James S, Snead DR, O'Hare JP, McTernan PG, Kumar S. Secretion of neuropeptide Y in human adipose tissue and its role in maintenance of adipose tissue mass. *Am J Physiol* 2007;**293**:E1335–40

51 Yang K, Guan H, Arany E, Hill DJ, Cao X. Neuropeptide Y is produced in visceral adipose tissue and promotes proliferation of adipocyte precursor cells via the Y1 receptor. *FASEB J* 2008; **22**:2452–64