Published in final edited form as:

Peptides. 2009 April ; 30(4): 715–720. doi:10.1016/j.peptides.2008.12.009.

Sympathetic nervous system-targeted neuropeptide Y overexpression in mice enhances neointimal formation in response to vascular injury

Suvi T. Ruohonen^{a,b,d}, Ken Abe^b, Mia Kero^c, Laura Toukola^a, Saku Ruohonen^a, Matias Rytt^c, Markku Koulu^a, Ullamari Pesonen^a, Zofia Zukowska^b, and Eriika Savontaus^{a,e,*}

^aDepartment of Pharmacology, Drug Development and Therapeutics, University of Turku, Finland

^bDepartment of Physiology, Biophysics and Neurosciences, Georgetown University Medical Center, Washington, DC, USA ^cDepartment of Pathology, University of Turku, Finland ^dDrug Discovery Graduate School, University of Turku, Finland ^eClinical Pharmacology, TYKSLAB, Health Care District of Southwest Finland, Finland

Abstract

Sympathetic neurotransmitter neuropeptide Y (NPY) is associated with vascular remodelling, neointimal hyperplasia and atherosclerosis in experimental animal models and clinical studies. In order to study the role of sympathetic nerve-produced NPY in vascular diseases, transgenic mouse model overexpressing NPY in central and peripheral noradrenergic neurons under the dopamine-beta-hydroxylase (DBH) promoter was recently created (OE-NPY^{DBH} mouse). This study aimed to examine the effect of NPY overexpression on arterial neointimal hyperplasia in an experimental model of vascular injury. Transgenic OE-NPY^{DBH} mice and wildtype control mice of two different inbred strains (C57BL/6 and FVB/n) underwent a femoral artery surgery with a transluminal injury by a 0.38-mm guide wire insertion. Arteries were harvested 4 weeks from the surgery, and they were stained for basic morphology. Both strains of OE-NPY^{DBH} mice, as compared with wildtype control mice, showed on average 50% greater formation of the neointima ($P < 0.01$) and an increase in the medial area ($P = 0.05$). The results suggest that moderately increased neuronal NPY causes the arteries to be more susceptible to femoral artery thickening after endothelial injury. The OE-NPY^{DBH} mouse provides a novel tool to explore the role of NPY in the development of vascular disease related to metabolic disorders.

Keywords

Neuropeptide Y; Transgenic mouse; Vascular injury; Neointimal hyperplasia

1. Introduction

Neuropeptide Y (NPY) is a 36-amino acid peptide present in sympathetic nervous system (SNS) co-localized with norepinephrine (NE) [6]. In humans, a functional leucine 7 to proline (p.L7P) polymorphism in the signal peptide of the *NPY* gene has been found [13]. This polymorphism is associated with cardio- and microvascular diseases such as

atherosclerosis and myocardial stroke [14,28,39], and diabetic retinopathy [11,17,29]. Clinical studies have also shown that healthy p.L7P subjects display increased levels of NPY in plasma during and after a treadmill exercise when compared with carriers of leucine 7 leucine (L7L) wildtype allele [12]. These data combined suggest that excess release of NPY from SNS supplying the blood vessels may be responsible for the faster progression of vascular diseases.

The idea of sympathetic transmitters controlling the vascular remodelling is generally accepted, but the mechanisms are not well known. It is acknowledged that SNS has trophic effects on vascular smooth muscle cells (VSMCs) in vivo and in vitro, and that the possible transmitters mediating the growth are NE and NPY [7–9,43,48]. It has also been shown that prolonged stress and increased release of adrenergic transmitters induce vascular inflammation, and further development of atherosclerosis [1]. NE has gained most of the attention as the principal sympathetic transmitter. However, NPY is co-released with NE potentiating its actions [5,6], and NPY accounts for about 30% of the sympathetic nerve-mediated vasoconstriction in resistance vessels [10]. NPY alone has been shown to have VSMC growth-promoting effects, which occur at lower concentrations than NPY's vasoconstrictive effects [46]. Furthermore, at low physiological concentrations, NPY is also an angiogenic factor promoting vessel sprouting and adhesion, migration, proliferation, and capillary tube formation of endothelial cells shown both in vitro and in vivo [47]. Unlike NE, which is preferentially released under acute stress, NPY is released by intense and prolonged sympathetic activation [45]. Thus, NPY is a mitogenic factor released from SNS upon prolonged stress, and it may be an important factor contributing to vascular growth after injury.

In order to study the role of SNS NPY, we have created a transgenic mouse model overexpressing *NPY* and reporter gene *LacZ* in central and peripheral noradrenergic neurons under the dopamine-beta-hydroxylase (DBH) promoter [33]. DBH catalyzes the final step in the biosynthesis of NE in noradrenergic and adrenergic neurons. Adrenal medulla, brainstem and sympathetic ganglia are the classic locations of DBH expression. We have shown that the OE-NPY^{DBH} mice display a 1.3-fold increase in NPY in the adrenal glands and 1.8-fold in the brainstem in comparison with wildtype mice [33], but the transgene expression in peripheral sympathetic ganglia innervating the organs and vasculature has not yet been characterized. The OE-NPY^{DBH} mice display notable alterations in lipid and glucose metabolism without affecting energy intake and total body weights [33]. Furthermore, the OE-NPY^{DBH} mice show increased responses to stress, that are seen as stress-related hypertension and increased stress-induced NPY secretion [34]. These observations together with the clinical data from carriers of the p.L7P allele led us to hypothesize that vascular remodelling is altered in the OE-NPY^{DBH} mouse.

SNS targets VSMCs via adrenergic α - and β -receptors and NPY receptors of different subtypes [22,46]. Femoral artery innervation density is intermediate in comparison with other arteries [37]. In SNS, DBH is expressed in perikarya of sympathetic neurons located in sympathetic ganglia. The first aim of this study was to visualize DBH-driven transgene expression in peripheral sympathetic neurons by reporter gene analysis in two major sympathetic ganglia: the superior cervical ganglion and the superior mesenteric ganglion. The main objective of this study was to perform a femoral artery wire injury, and to study the endothelial denudation-induced artery restenosis in OE-NPY^{DBH} transgenic mice in comparison with wildtype controls using two inbred transgenic mouse strains. FVB/n strain has been reported to respond well to an arterial angioplasty-induced neointima formation, but there is contradictory data on the susceptibility of C57BL/6 strain to this particular type of vascular injury [18,35,40,41]. Hence, we also wanted to study whether NPY might

improve the susceptibility of C57BL/6 mice to the neointima formation and to compare the results between the strains.

2. Materials and methods

2.1. Animals

A full description of the generation of the OE-NPY^{DBH} mouse model can be found elsewhere [33]. Briefly, a 10.6-kilobasepair transgene construct containing a promoter of the human *DBH* gene, a mouse *NPY* cDNA, an internal ribosomal entry site (IRES) sequence, a bacterial *LacZ* reporter gene and a simian virus poly-A sequence was microinjected into oocyte pronuclei isolated from a fertilized FVB/n mouse to generate hemizygous offspring. Eventually, the transgenic mouse on FVB/n background was backcrossed to C57BL/6 strain for 6 generations. Three-month-old male mice of both FVB/n and C57BL/6 background ($n = 8-9$ per group) were used in this study. Wildtype littermates of both strains were used as controls. The mice were anesthetized with ketamine (Ketalar® 75 mg/kg i.p.) and medetomidine (Domitor® 1 mg/kg i.p.) during surgical procedures and blood removal at sacrifice. The mice were kept in an animal room maintained at 21 ± 1 °C with a fixed 12:12 h light–dark cycle. Standard rodent chow and water were available ad libitum. All animal experiments were conducted with the approval of The Lab-Animal Care & Use Committee at the University of Turku, and the procedures were carried out according to institutional policies.

2.2. Sympathetic ganglia bacterial β -galactosidase expression

The superior cervical ganglia and the superior mesenteric ganglia were collected from untreated C57BL/6 mice of both genotypes. The ganglia were snap-frozen in liquid nitrogen and cut on gelatine-coated microscopic slides (20 μ m sections). Frozen sections were stained for bacterial β -galactosidase as previously described [33] to show transgene expression in SNS.

2.3. Mouse femoral artery angioplasty

Surgery was carried out under a dissecting microscope. The wire injury was performed as described earlier [35] on the femoral artery located on the right side of the body. A straight spring wire (0.38 mm in diameter, Cat. no. C-SF-15-15, Cook, Bloomington, IN) was used to remove the endothelial cell layer. The animals were given atipamezole (Antisedan® 1mg/kg) for a faster recovery from anesthesia. The mice were sacrificed exactly 4 weeks after surgery. Circulating blood was collected from abdominal vena cava before the femoral artery was carefully excised, perfused with 0.9% saline, and fixed in phosphate-buffered formalin overnight at 4 °C. Unoperated control vessels were obtained from contralateral femoral arteries. After fixation, vessels were placed in 70% ethanol and stored at 4 °C until embedded in paraffin.

2.4. Femoral artery morphometric analysis

Cross-sections of 5 μ m were stained with hematoxylin and eosin (H&E) and Masson's trichrome (MT), and imaged with an Olympus DP70 digital camera (Olympus America Inc., Center Valley, PA) on a Leica DMR microscope (Leica Microsystems GmbH, Wetzlar, Germany). For morphometry, neointimal hyperplasia was measured from the luminal side of the internal elastic lamina, and the media as an area between the external and the internal lamina. Areas were calculated by using a Cell*A imaging software (Soft Imaging System GmbH, Münster, Germany). The researcher was blind to the genotypes when selecting sections for analysis. Neointima and medial areas were averaged from consecutive 3 to 6

sections cut along the length of the injured area, where most notable neointima formation was observed.

2.5. Statistical analyses

The results are presented as means $\pm$ S.E.M. Statistical analyses were carried out using GraphPad Prism 4.03 (GraphPad Software Inc., San Diego, CA). Comparisons of the neointimal and medial areas were analyzed by using two-way analysis of variance (ANOVA) between genotypes and strains, or by Student's unpaired *t*-test between the genotypes. A statistical significance was reached at $P < 0.05$.

3. Results

3.1. Transgene expression in sympathetic ganglia

DBH promoter drives the expression of both transgenes: *NPY* and *LacZ*, which are transcribed into a single mRNA, but translated into two separate proteins due to the IRES sequence in between the genes [26]. Thus, bacterial β -galactosidase staining can be used to visualize the transgene expression in tissues. Clear blue color was detected in the sympathetic ganglia of transgenic but not wildtype sections (Fig. 1).

3.2. Mouse femoral artery injury model

All the mice survived the surgery and the healing of the wounds occurred rapidly. One of the mice (C57BL/6 strain) suffered from an injury to the femoral nerve resulting in a self-amputation of the operated limb. This animal was eliminated from the study. Of all the vessels, 2 out of 16 and 4 out of 17 (FVB/n and C57BL/6 strains, respectively) were discarded from the calculation of results due to an unsuccessful cutting and mounting of the sections. A marked neointima formation in the femoral artery was seen 4 weeks after the surgery in all the vessels studied, whereas no changes were seen in the contralateral uninjured arteries (Fig. 2). Lumen and medial areas (normalized for body weights) of uninjured arteries were similar between the genotypes (data not shown).

3.3. Effect of NPY overexpression on neointima and media thickening

The neointima formation calculated as a lesion area inside the internal elastic lamina was more pronounced in both strains of the OE-NPY^{DBH} mice than in wildtype control mice (genotype: $P < 0.01$, strain: $P = \text{NS}$, genotype $\times$ strain interaction: $P = \text{NS}$, two-way ANOVA, Fig. 3A). The OE-NPY^{DBH} mice also appeared to experience more severe growth of the medial area after the injury than did the wildtype mice (genotype: $P = 0.05$, strain: $P = \text{NS}$, genotype $\times$ strain interaction: $P = \text{NS}$, two-way ANOVA, Fig. 3B). Thus, the background strain did not seem to affect the response to the injury. In the OE-NPY^{DBH} mice (analyzed in C57BL/6), the procedure significantly increased the arterial medial area from the uninjured contralateral side, but no such changes were observed in wildtype controls ($P < 0.05$, Student's *t*-test, Fig. 4).

4. Discussion

This study shows that overexpression of *NPY* in sympathetic nerves significantly promotes femoral artery thickening by neointima formation and medial layer hypertrophy after an arterial endovascular dilatation injury in the OE-NPY^{DBH} mice compared with wildtype control mice in FVB/n and C57BL/6 background strains. Both strains were equally responsive to the injury in wildtype controls, and thus, the mouse background did not affect the outcome. Overexpression of *NPY* in the OE-NPY^{DBH} mice is driven by the previously described DBH promoter in noradrenergic and adrenergic neurons [15,16,24]. We have earlier shown that the *NPY* overexpression in adrenal chromaffin cells and NE-rich brain

areas leads to 1.3–1.8-fold increase in NPY protein levels [33]. Here we show that the LacZ transgene (co-expressed with NPY) is produced in the sympathetic ganglia, the origin of sympathetic innervation to internal organs. Femoral artery, the artery injured in this study, contains a dense plexus of vascular noradrenergic nerve terminals at the adventitial-medial border deriving from sympathetic ganglion. Transgenic NPY produced in the ganglia is delivered to nerve terminals by axonal transport and co-released with NE upon sympathetic activation.

Increased neointima formation in the OE-NPY^{DBH} mice is in line with previous work on the effects of NPY in the endovascular injury model [18–20]. In rats, a local periarterially inserted NPY pellet dose-dependently increased the formation of neointima and medial areas, which was prevented by continuous infusion of NPY receptor Y₁ or Y₅ antagonists [20]. A similar effect was observed in rats exposed to mild chronic stress leading to increased endogenous NPY release from the SNS [19]. In contrast to the previous studies, we observed a notable increase in the arterial remodelling in the presence of near physiological overexpression of NPY in the sympathetic nerves instead of pharmacological dosing of NPY or environmental intervention, that inevitably lead to multiple significant changes in other neurotransmitters and growth factors. NPY has been shown to increase DNA synthesis and cell proliferation rate in a dose- and cell density-dependent manner with up to 20-fold potency over NE in rat, porcine and human aortic VSMC lines [27,36,46]. In the most recent study, it was shown that NPY-mediated VSMC mitogenesis signals primarily via Y₁ receptors activating two Ca²⁺-dependent growth-promoting pathways: protein kinase C and calcium/calmodulin-dependent kinase II [32] that can be attenuated by incubation with calcium channel blockers [46]. These two pathways are amplified by the third Y₅-mediated calcium-independent inhibition of the adenylyl cyclase protein kinase A pathway. Eventually, all three mechanisms converge to the extracellular signal-regulated kinase signaling cascade and lead to VSMC proliferation [32]. Thus, NPY acts directly on VSMCs to promote proliferation, and this is likely to be a key element of NPY-induced vascular wall thickening, and could be hypothesized to be prevented using Y₁ or Y₅ receptor antagonists.

In addition to NPY's direct effects on the vasculature, metabolic factors might contribute to increased vascular hypertrophy in the OE-NPY^{DBH} mice, as the mice show an abnormal metabolic profile. The OE-NPY^{DBH} mice display increased white adipose tissue mass and hepatic lipid accumulation already at the age of 3 months, but do not show hyperphagia, increased weight gain, or decreased locomotor activity [33]. Increased adiposity leads to impaired glucose tolerance later in life [33]. We have also shown stress-increased arterial blood pressure responses in the OE-NPY^{DBH} mice measured with radiotelemetry in conscious animals [34]. Overall, the OE-NPY^{DBH} mice display several traits of the human metabolic syndrome. Other mouse models of metabolic disorders, such as apoE deficient and LDL receptor null mice, also show increased neointima formation after angioplasty similar to what we have observed here [4,23,44]. However, leptin deficient and leptin receptor deficient obese mice (ob/ob and db/db mice, respectively), display decreased neointima formation. Administration of leptin to ob/ob mice significantly increases the arterial inward remodelling process [2,3,38]. These data suggest that altered blood lipid levels and leptin are responsible for the arterial hypertrophy either by increasing the inflammatory process in the vascular wall, or by directly activating the leptin receptors in VSMCs. Although, the OE-NPY^{DBH} mice show increased adiposity at 3 months, plasma leptin levels are not significantly increased compared with wildtype mice [33]. Blood levels of glucose, cholesterol and triglyceride are also normal in young OE-NPY^{DBH} mice [33]. Furthermore, hypertension is not observed at baseline but during recovery from the telemetry probe implantation surgery [34]. Thus, leptin, impaired glucose metabolism,

increased blood lipids, or hypertension do not appear to be important factors in vascular remodelling in the OE-NPY^{DBH} model.

Humans with *NPY* gene L7P polymorphism and the OE-NPY^{DBH} mouse both display exaggerated NPY release from the SNS. We have earlier shown that the p.L7P allele is associated with vascular diseases, i.e. accelerated neointima formation and earlier onset of atherosclerosis independent of other risk factors [14] and development of diabetic retinopathy in type 2 diabetic patients [29]. The polymorphism is also associated with metabolic factors increasing the risk for vascular disease such as increased serum LDL [13] and an earlier onset of type 2 diabetes in obese carriers of the p.L7P allele [11]. However, the L7P polymorphism did not associate with a higher rate of coronary stent restenosis after a percutaneous intervention [31]. The data from the OE-NPY^{DBH} mouse show that increased NPY in sympathetic nerves and brain noradrenergic neurons leads to increased adiposity, impaired glucose tolerance and increased susceptibility to arterial thickening after vascular injury, and provides further support to the hypothesis that excess NPY release in humans leads to metabolic disturbances and vascular disease as seen in carriers of the p.L7P allele. What remains to be determined is whether blockade of sympathetic tone with, e.g. β -blockers or angiotensin-converting enzyme (ACE) inhibitors will ameliorate the metabolic and/or vascular phenotype in the OE-NPY^{DBH} model. It has been shown in rats and men that ACE inhibitors decrease plasma NPY and NE levels by preventing the angiotensin II-induced sympathetic transmitter release via presynaptic angiotensin receptors [21,25,42]. β -Blockers, on the other hand, had no effect on plasma NPY levels in normo- or hypertensive rats [42], or on plasma catecholamine levels in humans under stress [30].

5. Conclusions

In conclusion, as compared with wildtype control mice, the OE-NPY^{DBH} mice display on average 50% greater formation of arterial neointima, and a 30% increase in the medial area following an arterial angioplasty surgery in two different inbred mouse strains. We suggest that locally increased SNS NPY is responsible for the hypertrophic effect. Hence, increased sympatho-adrenomedullary-released NPY seems to be an important risk factor for vascular diseases related to the metabolic syndrome. The OE-NPY^{DBH} mouse model allows further exploration of the role of NPY in the development of this complex disorder.

Nomenclature

DBH	dopamine-beta-hydroxylase
NE	norepinephrine
NPY	neuropeptide Y
p.L7P	leucine 7 to proline polymorphism
SNS	sympathetic nervous system
VSMC	vascular smooth muscle cell

Acknowledgments

The authors would like to thank Ms. Sanna Martti, Miss Pipsa Lappalainen and Mr. Jouko Sandholm (Cell Imaging Core, Turku Centre for Biotechnology) for skillful technical assistance. Ms. Pirkko Huuskonen is acknowledged for reviewing of the language. This study was supported by the Academy of Finland, the Finnish Cultural Foundation and the Turku University Foundation.

References

1. Black PH. The inflammatory consequences of psychologic stress: relationship to insulin resistance, obesity, atherosclerosis and diabetes mellitus, type II. *Med Hypotheses* 2006;67(4):879–891. [PubMed: 16781084]
2. Bodary PF, Shen Y, Ohman M, Bahrou KL, Vargas FB, Cudney SS, et al. Leptin regulates neointima formation after arterial injury through mechanisms independent of blood pressure and the leptin receptor/STAT3 signaling pathways involved in energy balance. *Arterioscler Thromb Vasc Biol* 2007;27(1):70–76. [PubMed: 17095713]
3. Bodary PF, Westrick RJ, Wickenheiser KJ, Shen Y, Eitzman DT. Effect of leptin on arterial thrombosis following vascular injury in mice. *JAMA* 2002;287(13):1706–1709. [PubMed: 11926895]
4. Chadjichristos CE, Matter CM, Roth I, Sutter E, Pelli G, Luscher TF, et al. Reduced connexin43 expression limits neointima formation after balloon distension injury in hypercholesterolemic mice. *Circulation* 2006;113(24):2835–2843. [PubMed: 16769907]
5. Edvinsson L. Characterization of the contractile effect of neuropeptide Y in feline cerebral arteries. *Acta Physiol Scand* 1985;125(1):33–41. [PubMed: 2864784]
6. Ekblad E, Edvinsson L, Wahlestedt C, Uddman R, Hakanson R, Sundler F. Neuropeptide Y co-exists and co-operates with noradrenaline in perivascular nerve fibers. *Regul Pept* 1984;8(3):225–235. [PubMed: 6379758]
7. Erami C, Zhang H, Ho JG, French DM, Faber JE. Alpha(1)-adrenoceptor stimulation directly induces growth of vascular wall in vivo. *Am J Physiol Heart Circ Physiol* 2002;283(4):H1577–H1578. [PubMed: 12234812]
8. 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(3):259–265. [PubMed: 8016410]
9. Erlinge D, Yoo H, Edvinsson L, Reis DJ, Wahlestedt C. Mitogenic effects of ATP on vascular smooth muscle cells vs. other growth factors and sympathetic cotransmitters. *Am J Physiol* 1993;265(4 Pt 2):H1089–H1097. [PubMed: 7694483]
10. 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(1 Pt 2):H290–H294. [PubMed: 9458879]
11. Jaakkola U, Pesonen U, Vainio-Jylha E, Koulu M, Pollonen M, Kallio J. The Leu7Pro polymorphism of neuropeptide Y is associated with younger age of onset of type 2 diabetes mellitus and increased risk for nephropathy in subjects with diabetic retinopathy. *Exp Clin Endocrinol Diabetes* 2006;114(4):147–152. [PubMed: 16705546]
12. Kallio J, Pesonen U, Kaipio K, Karvonen MK, Jaakkola U, Heinonen OJ, et al. Altered intracellular processing and release of neuropeptide Y due to leucine 7 to proline 7 polymorphism in the signal peptide of preproneuropeptide Y in humans. *Faseb J* 2001;15(7):1242–1244. [PubMed: 11344101]
13. Karvonen MK, Pesonen U, Koulu M, Niskanen L, Laakso M, Rissanen A, et al. Association of a leucine(7)-to-proline(7) polymorphism in the signal peptide of neuropeptide Y with high serum cholesterol and LDL cholesterol levels. *Nat Med* 1998;4(12):1434–1437. [PubMed: 9846584]
14. Karvonen MK, Valkonen VP, Lakka TA, Salonen R, Koulu M, Pesonen U, et al. 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(1):145–151. [PubMed: 11689216]
15. Kobayashi K, Morita S, Mizuguchi T, Sawada H, Yamada K, Nagatsu I, et al. Functional and high-level expression of human dopamine beta-hydroxylase in transgenic mice. *J Biol Chem* 1994;269(47):29725–29731. [PubMed: 7961964]
16. Kobayashi K, Sasaoka T, Morita S, Nagatsu I, Iguchi A, Kurosawa Y, et al. Genetic alteration of catecholamine specificity in transgenic mice. *Proc Natl Acad Sci USA* 1992;89(5):1631–1635. [PubMed: 1542654]

17. Koulou M, Movafagh S, Tuohimaa J, Jaakkola U, Kallio J, Pesonen U, et al. Neuropeptide Y and Y2-receptor are involved in development of diabetic retinopathy and retinal neovascularization. *Ann Med* 2004;36(3):232–240. [PubMed: 15181979]
18. Kuo LE, Abe K, Zukowska Z. Stress, NPY and vascular remodeling: implications for stress-related diseases. *Peptides* 2007;28(2):435–440. [PubMed: 17241699]
19. 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(10):2075–2080. [PubMed: 16051880]
20. 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(7):1204–1210. [PubMed: 12689918]
21. Majewski H, Hedler L, Schurr C, Starke K. Modulation of noradrenaline release in the pithed rabbit: a role for angiotensin II. *J Cardiovasc Pharmacol* 1984;6(5):888–896. [PubMed: 6209496]
22. Malmstrom RE. Neuropeptide Y Y1 receptor mediated mesenteric vasoconstriction in the pig in vivo. *Regul Pept* 2000;95(1–3):59–63. [PubMed: 11062333]
23. Matter CM, Ma L, von Lukowicz T, Meier P, Lohmann C, Zhang D, et al. Increased balloon-induced inflammation, proliferation, and neointima formation in apolipoprotein E (ApoE) knockout mice. *Stroke* 2006;37(10):2625–2632. [PubMed: 16946151]
24. Mercer EH, Hoyle GW, Kapur RP, Brinster RL, Palmiter RD. The dopamine beta-hydroxylase gene promoter directs expression of *E. coli lacZ* to sympathetic and other neurons in adult transgenic mice. *Neuron* 1991;7(5):703–716. [PubMed: 1742021]
25. Mills PJ, Dimsdale JE, Ziegler MG, Hauger RL, Nelesen RA, Brown MR. Sympathetic alterations after sodium restriction and short-term captopril administration. *J Am Coll Cardiol* 1993;21(1):177–181. [PubMed: 8380185]
26. Mountford PS, Smith AG. Internal ribosome entry sites and dicistronic RNAs in mammalian transgenesis. *Trends Genet* 1995;11(5):179–184. [PubMed: 7540337]
27. Nilsson T, Edvinsson L. Neuropeptide Y stimulates DNA synthesis in human vascular smooth muscle cells through neuropeptide Y Y1 receptors. *Can J Physiol Pharmacol* 2000;78(3):256–259. [PubMed: 10721818]
28. Niskanen L, Karvonen MK, Valve R, Koulou M, Pesonen U, Mercuri M, et al. Leucine 7 to proline 7 polymorphism in the neuropeptide Y gene is associated with enhanced carotid atherosclerosis in elderly patients with type 2 diabetes and control subjects. *J Clin Endocrinol Metab* 2000;85(6):2266–2269. [PubMed: 10852461]
29. Niskanen L, Voutilainen-Kaunisto R. Leucine 7 to proline 7 polymorphism in the neuropeptide y gene is associated with retinopathy in type 2 diabetes. *Exp Clin Endocrinol Diabetes* 2000;108(3):235–236. [PubMed: 10926322]
30. Paran E, Neumann L, Cristal N. Effects of mental and physical stress on plasma catecholamine levels before and after beta-adrenoceptor blocker treatment. *Eur J Clin Pharmacol* 1992;43(1):11–15. [PubMed: 1354617]
31. Pesonen U, Koch W, Schomig A, Kastrati A. Leucine 7 to proline 7 polymorphism of the preproneuropeptide Y gene is not associated with restenosis after coronary stenting. *J Endovasc Ther* 2003;10(3):566–572. [PubMed: 12932169]
32. Pons J, Kitlinska J, Jacques D, Perreault C, Nader M, Everhart L, et al. Interactions of multiple signaling pathways in neuropeptide Y-mediated bimodal vascular smooth muscle cell growth. *Can J Physiol Pharmacol* 2008;86(7):438–448. [PubMed: 18641693]
33. Ruohonen ST, Pesonen U, Moritz N, Kaipio K, Roytta M, Koulou M, et al. Transgenic mice overexpressing neuropeptide Y in noradrenergic neurons: a novel model of increased adiposity and impaired glucose tolerance. *Diabetes* 2008;57(6):1517–1525. [PubMed: 18276767]
34. Ruohonen ST, Savontaus E, Rinne P, Rosmaninho-Salgado J, Cavadas C, Ruskoaho H, Koulou M, Pesonen U. Stress-induced hypertension and increased sympathetic activity in mice overexpressing neuropeptide Y in noradrenergic neurons. *Neuroendocrinology*. 2008 doi: [10.1159/000188602](https://doi.org/10.1159/000188602), in press.

35. Sata M, Maejima Y, Adachi F, Fukino K, Saiura A, Sugiura S, et al. A mouse model of vascular injury that induces rapid onset of medial cell apoptosis followed by reproducible neointimal hyperplasia. *J Mol Cell Cardiol* 2000;32(11):2097–2104. [PubMed: 11040113]
36. Shigeri Y, Fujimoto M. Neuropeptide Y stimulates DNA synthesis in vascular smooth muscle cells. *Neurosci Lett* 1993;149(1):19–22. [PubMed: 8469373]
37. Stassen FR, Maas RG, Schiffers PM, Janssen GM, De Mey JG. A positive and reversible relationship between adrenergic nerves and alpha-1A adrenoceptors in rat arteries. *J Pharmacol Exp Ther* 1998;284(1):399–405. [PubMed: 9435203]
38. Stephenson K, Tunstead J, Tsai A, Gordon R, Henderson S, Dansky HM. Neointimal formation after endovascular arterial injury is markedly attenuated in db/db mice. *Arterioscler Thromb Vasc Biol* 2003;23(11):2027–2033. [PubMed: 14500292]
39. Wallerstedt SM, Skrtic S, Eriksson AL, Ohlsson C, Hedner T. Association analysis of the polymorphism T1128C in the signal peptide of neuropeptide Y in a Swedish hypertensive population. *J Hypertens* 2004;22(7):1277–1281. [PubMed: 15201542]
40. Wang X, Chai H, Lin PH, Lumsden AB, Yao Q, Chen C. Mouse models of neointimal hyperplasia: techniques and applications. *Med Sci Monit* 2006;12(9):RA177–RA185. [PubMed: 16940942]
41. Wang X, Paigen B. Comparative genetics of atherosclerosis and restenosis: exploration with mouse models. *Arterioscler Thromb Vasc Biol* 2002;22(6):884–886. [PubMed: 12067893]
42. Zeng C, Wang X, Liu G, Yang C. Effects of ACE inhibitor and beta-adrenergic blocker on plasma NPY and NPY receptors in aortic vascular smooth muscle cells from SHR and WKY rats. *Neuropeptides* 2002;36(5):353–361. [PubMed: 12450741]
43. Zhang H, Facemire CS, Banas AJ, Faber JE. Different alpha-adrenoceptors mediate migration of vascular smooth muscle cells and adventitial fibroblasts in vitro. *Am J Physiol Heart Circ Physiol* 2002;282(6):H2364–H2370. [PubMed: 12003847]
44. Zhu B, Kuhel DG, Witte DP, Hui DY. Apolipoprotein E inhibits neointimal hyperplasia after arterial injury in mice. *Am J Pathol* 2000;157(6):1839–1848. [PubMed: 11106557]
45. Zukowska-Grojec Z, Neuropeptide Y. A novel sympathetic stress hormone and more. *Ann N Y Acad Sci* 1995;771:219–233. [PubMed: 8597401]
46. Zukowska-Grojec Z, Karwatowska-Prokopczuk E, Fisher TA, Ji H. Mechanisms of vascular growth-promoting effects of neuropeptide Y: role of its inducible receptors. *Regul Pept* 1998;75–76: 231–238.
47. Zukowska-Grojec Z, Karwatowska-Prokopczuk E, Rose W, Rone J, Movafagh S, Ji H, et al. Neuropeptide Y: a novel angiogenic factor from the sympathetic nerves and endothelium. *Circ Res* 1998;83(2):187–195. [PubMed: 9686758]
48. Zukowska-Grojec Z, Pruszczyk P, Colton C, Yao J, Shen GH, Myers AK, et al. Mitogenic effect of neuropeptide Y in rat vascular smooth muscle cells. *Peptides* 1993;14(2):263–268. [PubMed: 8483806]

Fig. 1.

LacZ transgene expression in OE-NPY^{DBH} and wildtype sympathetic ganglia. The superior cervical ganglia (SCG) and the superior mesenteric ganglia (SMG) were harvested from adult untreated OE-NPY^{DBH} and wildtype (WT) C57BL/6 male mice. Frozen sections were stained for bacterial β -galactosidase and counterstained with Nuclear Fast Red to visualize the expression of transgenic *LacZ* reporter gene. Clear blue color is observed in both ganglia from OE-NPY^{DBH} mice but not in any wildtype sections. The outlined area is imaged with greater (20 $\times$) magnification in lower panel. Wildtype sections are imaged with 20 $\times$ magnification. Scale bar is 200 μ m. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Fig. 2. Neointima formation after femoral artery wire injury. Hematoxylin & eosin (left panel) and Masson's trichrome (right panel) staining of the wildtype (WT) and OE-NPY^{DBH} injured arteries with marked neointimal lesions at 4 weeks from the surgery compared with an uninjured control artery (FVB/n strain). Black dotted arrow = an inner boundary of the neointima (NI); Black arrow = the internal elastic lamina (IE); White arrow = the external elastic lamina (EE). IE–NI = neointimal area; EE–IE = medial area. Scale bar is 100 μ m.

Fig. 3. Calculations of arterial neointima and medial areas. (A) Average neointima area after injury in FVB/n (wildtype: $n = 7$; OE-NPY^{DBH}: $n = 7$) and C57BL/6 mice (wildtype: $n = 5$; OE-NPY^{DBH}: $n = 8$). (B) Average medial area after injury in FVB/n and C57BL/6 mice (for n see A). Values are expressed as means $\pm$ S.E.M. Statistical analyses were performed with two-way ANOVA.

Fig. 4. Medial area of injured vs. uninjured femoral arteries. Average medial area of uninjured (wildtype: $n = 5$; OE-NPY^{DBH}: $n = 5$) and injured (wildtype: $n = 5$; OE-NPY^{DBH}: $n = 8$) arteries measured in C57BL/6 mice. Values are expressed as means $\pm$ S.E.M. Statistical analyses were performed by Student's t -test.