Published in final edited form as:

J Sex Med. 2012 August ; 9(8): 1980–1987. doi:10.1111/j.1743-6109.2012.02798.x.

Targeting NADPH Oxidase Decreases Oxidative Stress in the Transgenic Sick Cell Mouse Penis

Biljana Musicki, PhD, Tongyun Liu, MS, Sena F. Sezen, PhD, and Arthur L. Burnett, MD
Department of Urology, The Johns Hopkins University, Baltimore, MD, USA

Abstract

Introduction—Sickle cell disease (SCD) is a state of chronic vasculopathy characterized by endothelial dysfunction and increased oxidative stress, but the sources and mechanisms responsible for reactive oxygen species (ROS) production in the penis are unknown.

Aims—We evaluated whether SCD activates NADPH oxidase, induces endothelial nitric oxide synthase (eNOS) uncoupling, and decreases antioxidants in the SCD mouse penis. We further tested the hypothesis that targeting NADPH oxidase decreases oxidative stress in the SCD mouse penis.

Methods—SCD transgenic (sickle) mice were used as an animal model of SCD. Hemizygous (hemi) mice served as controls. Mice received an NADPH oxidase inhibitor apocynin (10 mM in drinking water) or vehicle. Penes were excised at baseline for molecular studies. Markers of oxidative stress (4-hydroxy-2-nonenal [HNE]), sources of ROS (eNOS uncoupling and NADPH oxidase subunits p67^{phox}, p47^{phox}, and gp91^{phox}), and enzymatic antioxidants (superoxide

© 2012 International Society for Sexual Medicine

Corresponding Author: Biljana Musicki, PhD, Department of Urology, The Johns Hopkins University, 600 North Wolfe Street, Marburg 405, Baltimore, MD 21287, USA. Tel: (410) 955-0352; Fax: (410) 614-3695; bmusicki@jhmi.edu.

Conflict of Interest: None.

Statement of Authorship

Category 1

a. Conception and Design

Biljana Musicki; Arthur L. Burnett

b. Acquisition of Data

Biljana Musicki; Tongyun Liu; Sena F. Sezen

c. Analysis and Interpretation of Data

Biljana Musicki; Arthur L. Burnett

Category 2

a. Drafting the Article

Biljana Musicki

b. Revising It for Intellectual Content

Biljana Musicki; Tongyun Liu; Sena F. Sezen; Arthur L. Burnett

Category 3

a. (Final Approval of the Completed Article

Biljana Musicki; Tongyun Liu; Sena F. Sezen; Arthur L. Burnett

dismutase [SOD]1, SOD2, catalase, and glutathione peroxidase-1 [GPx1]) were measured by Western blot in penes.

Main Outcome Measures—Sources of ROS, oxidative stress, and enzymatic antioxidants in the SCD penis.

Results—Relative to hemi mice, SCD increased ($P < 0.05$) protein expression of NADPH oxidase subunits p67^{phox}, p47^{phox}, and gp91^{phox}, 4-HNE-modified proteins, induced eNOS uncoupling, and reduced Gpx1 expression in the penis. Apocynin treatment of sickle mice reversed ($P < 0.05$) the abnormalities in protein expressions of p47^{phox}, gp91^{phox} (but not p67^{phox}) and 4-HNE, but only slightly ($P > 0.05$) prevented eNOS uncoupling in the penis. Apocynin treatment of hemi mice did not affect any of these parameters.

Conclusion—NADPH oxidase and eNOS uncoupling are sources of oxidative stress in the SCD penis; decreased GPx1 further contributes to oxidative stress. Inhibition of NADPH oxidase upregulation decreases oxidative stress, implying a major role for NADPH oxidase as a ROS source and a potential target for improving vascular function in the SCD mouse penis.

Keywords

eNOS Uncoupling; Antioxidants; Priapism

Introduction

Sickle cell disease (SCD) is a hemoglobinopathy resulting from the expression of abnormal sickle hemoglobin (HbS) and consequent red blood cell rigidity, diminished blood flow, and hypoxia. SCD is also characterized by a chronic vasculopathy, involving reduced nitric oxide (NO) bioavailability, enhanced responses to vasoconstrictors, and elevated oxidative stress [1,2].

Oxidative stress, an imbalance between the production and elimination of reactive oxygen species (ROS), has been proposed to be a major factor in the pathogenesis of SCD. ROS, produced in vascular smooth muscle cells and endothelial cells, may induce vascular dysfunction by scavenging NO or affecting eNOS expression and activity, depleting NOS cofactors, generating vasoconstrictors, affecting smooth muscle cell integrity, inactivating antioxidants, and causing structural and functional changes [3]. Increased ROS production in SCD has been attributed to upregulation of NADPH oxidase [4], activation of xanthine oxidase [5], eNOS uncoupling [6,7], as well as autooxidation of HbS [1]. In addition, the protective mechanisms afforded by antioxidants are decreased in SCD. SCD patients and animal models exhibit low levels of vitamins A, C, E, and zinc [8,9] and deficiency of glutathione reductase [10] and glutathione peroxidase (GPx) [11], while superoxide dismutase (SOD) protein expression or activity and catalase expression are either decreased [12,13] or increased [14].

Major clinical manifestations of vasculopathy in SCD include pulmonary hypertension, priapism, and stroke. Priapism is a highly prevalent complication of SCD, affecting about 40% of men with SCD [15–17]. This erection disorder consists of non-willful, excessive, and often recurrent penile erection unrelated to sexual excitement or desire. Ischemic priapism, the most common form of priapism in which blood flow in the corpora cavernosa is absent, is frequently associated with irreversible penile fibrosis and tissue damage, and, if untreated, may lead to permanent and irreversible erectile dysfunction (ED) [18–20].

The mechanisms responsible for priapism are the focus of intense research, but still remain incompletely defined. Priapism has been associated with chronically impaired NO bioavailability in the penis resulting in a defect in the NO/cyclic guanosine monophosphate/

phosphodiesterase type 5 signaling pathway [16,21–23], overproduction of adenosine [18,24], and upregulation of opiorphins (enzymes involved in the polyamine synthesis) [25]. The NO depletion mechanism has been attributed to decreased eNOS phosphorylation on Ser-1177 and decreased eNOS interaction with heat-shock protein 90 [26].

Oxidative stress in the SCD penis contributes to endothelial dysfunction and cavernosal tissue damage. Increased lipid peroxidation and protein oxidation in corporal tissue of rats with opiorphin-induced priapism and in transgenic SCD mice have recently been reported [27]. However, the sources of ROS and the mechanisms responsible for ROS formation in the penis remain unknown.

Aims

The aim of this study was to investigate enzymatic sources of ROS, specifically NADPH oxidase and eNOS uncoupling, selected enzymatic antioxidants, and oxidative stress, in the SCD penis. We further tested the hypothesis that targeting NADPH oxidase decreases oxidative stress, conceivably improving vascular function in the SCD mouse penis. For this study, we used transgenic SCD mouse model.

Materials and Methods

Animals

Seven to 9 months old male transgenic SCD (sickle) and hemizygous (hemi) mice were used [19]. Transgenic SCD mice with knockout of all mouse hemoglobin genes and expressing exclusively human HbS were developed at Lawrence Berkeley National Laboratory [28]. Sickle and hemi mice are obtained by breeding sickle male mice to hemi females in-house. Sickle and hemi mice were treated with an NADPH oxidase inhibitor apocynin (10 mM, 8.3 mg/day in drinking water, Calbiochem, San Diego, CA, USA) or vehicle for 4 weeks [29]. Flaccid penes were collected before hemi (N = 14) and sickle (N = 14) mice were sacrificed. All experiments were conducted in accordance with the ethical standards of the Johns Hopkins University School of Medicine Guidelines for the Care and Use of Animals.

Western Blot Analysis

Minced penile tissue was homogenized as described [30]. Homogenates were resolved on 4–20% Tris gels and transferred to polyvinylidene difluoride membrane. Membranes were probed with polyclonal rabbit anti-4-hydroxy-2-nonenal (HNE) antibody at 1:2,000 (Alpha Diagnostic International, San Antonio, TX, USA), rabbit anti-p47^{phox} antibody at 1:1,000 (Upstate Biotechnology Inc., Lake Placid, NY, USA), mouse anti-p67^{phox} antibody at 1:600, mouse anti-gp91^{phox} antibody at 1:1,000 (both from BD Transduction Laboratories, San Diego, CA, USA) [29], or rabbit anti-SOD1, SOD2, GPx1, or catalase antibodies (all from Abcam, Cambridge, MA, USA) at 1:1,000 dilutions. Signals were standardized to β -actin. For the analysis of the dimeric and monomeric forms of eNOS, low-temperature sodium dodecyl sulfate (SDS)-gel electrophoresis was used with partially purified penile homogenates, as described previously [31]. Membranes were then probed with rabbit anti-eNOS antibody (BD Transduction Laboratories) at 1:1,000 dilution. Bands were detected by horseradish peroxidase conjugated anti-mouse or anti-rabbit antibodies (GE Health-care, Piscataway, NJ, USA), and analyzed using National Institutes of Health Image software. eNOS uncoupling was represented inversely as a ratio of active eNOS dimers to inactive eNOS monomers. All results were expressed relative to hemi mice treated with vehicle.

Statistical Analysis

Statistical analysis was performed by using one-way analysis of variance, followed by Newman–Keuls multiple comparison test or by *t*-test when appropriate. The data were expressed as the mean $\pm$ standard error of the mean. A value of $P < 0.05$ was considered to be statistically significant.

Results

Effect of SCD on Protein Expression of Antioxidants GPx1, SOD1, SOD2, and Catalase in the Penis

Protein expression of GPx1, a key antioxidant enzyme, was significantly ($P < 0.05$) decreased in penes of sickle compared to hemi mice. Protein expression of SOD1, SOD2, and catalase did not differ in penes of sickle compared to hemi mice (Figure 1).

Effect of SCD and Apocynin Treatment on Protein Expression of NADPH Oxidase Subunits in the Penis

Protein expressions of NADPH oxidase subunits p67^{phox}, p47^{phox}, and gp91^{phox} were significantly ($P < 0.05$) increased in penes of sickle compared to hemi mice. Treatment of sickle mice with apocynin, which inhibits the assembly of the subunits within the membrane to an active enzymatic complex and decreases protein expression of NADPH oxidase subunits [29,32], significantly ($P < 0.05$) decreased protein expressions of p47^{phox} and gp91^{phox} (but not p67^{phox}) in the penis (Figure 2). Apocynin treatment of hemi mice did not affect protein expressions of any of the NADPH oxidase subunits in the penis.

Effect of SCD and Apocynin Treatment on eNOS Uncoupling in the Penis

The ratio of eNOS dimer (functional eNOS)/monomer (nonfunctional eNOS), inversely related to eNOS uncoupling, was significantly ($P < 0.05$) decreased in penes of sickle mice relative to levels found in hemi mouse penes (Figure 3). Apocynin treatment slightly, but not significantly ($P > 0.05$), increased the dimer/monomer ratio in penes of sickle mice, and had no effect on this ratio in penes of hemi mice.

Effect of SCD and Apocynin Treatment on 4-HNE-Modified Proteins in the Penis

4-HNE is a relatively stable end product of lipid peroxidation used as a biomarker for oxidative damage. It is membrane diffusible and highly reactive, and covalently binds to histidine, lysine, and cysteine residue of proteins, forming adducts that alter protein function and structure [33]. The amount of 4-HNE-modified proteins was significantly ($P < 0.05$) increased in penes of sickle mice compared with the pattern in hemi mice (Figure 4). Apocynin significantly ($P < 0.05$) decreased 4-HNE expression in the penis of sickle mice, and had no effect on 4-HNE expression in penes of hemi mice.

Discussion

The present study demonstrates that NADPH oxidase and eNOS uncoupling are sources of ROS in the penis of sickle mice. Deficiency of GPx1 further contributes to oxidative stress by weakening an enzymatic antioxidant potential. Targeting NADPH oxidase decreases oxidative stress in SCD penis, implying a major role for NADPH oxidase as a source of ROS in the SCD penis. Taken together, our data suggest that increased oxidative stress due to upregulation of NADPH oxidase and eNOS uncoupling, and decreased antioxidant GPx1 in the SCD penis, conceivably provides a molecular basis for SCD-associated erectile disorder. The findings further suggest that targeting NADPH oxidase may offer a potential treatment for improving vascular function in the SCD penis, and, conceivably, priapism.

This is of special importance, since recent studies point to the greater importance of targeting ROS formation, rather than ROS scavenging, in reducing oxidative damage [34].

Oxidative stress plays a significant role in SCD, contributing to endothelial dysfunction. Activated endothelial cell NADPH oxidase has been implicated in ROS production in the cerebral vasculature of SCD transgenic mice [4]. The NADPH oxidases are a family of enzymes that catalyze electron transfer from cytosolic NADPH or NADH to molecular oxygen to generate superoxide. Of the seven family members, four have been identified as important sources of ROS in the vasculature: Nox1, Nox2, Nox4, and Nox5. The prototype Nox2 (gp91^{phox})-containing NADPH oxidase possesses cytosolic subunits (p47^{phox}, p67^{phox}, or homologues) and membrane-bound subunits (gp91^{phox} and p22^{phox}), which form a functional enzyme complex upon activation [35]. Increased protein expression of NADPH oxidase subunits has been demonstrated in the penis of diabetic [36,37], hypertensive [38,39], and hypercholesterolemic [29] animals in parallel with increased oxidative stress and ED. We now report that SCD is associated with increased protein expression of NADPH oxidase subunits p47^{phox}, p67^{phox}, and gp91^{phox} in the penis, implying activation of NADPH oxidase in the penis by SCD.

Uncoupled eNOS represents another important source of ROS that may contribute to SCD vasculopathy. Under physiologic conditions, NOS isoforms structurally reside as homodimeric oxidoreductases. This form permits their oxidative catalysis of L-arginine to NO. In the uncoupled state, electron transfer within the active site of eNOS becomes “uncoupled” from L-arginine oxidation, and results in oxidation of molecular oxygen and preferential superoxide production over NO production [40]. Moreover, superoxide is rapidly converted into highly toxic nitrogen species such as peroxynitrite, if NO is also present locally. Peroxynitrite causes cellular dysfunction through oxidative damage to DNA, proteins and lipids, nitration of tyrosines, release of vasoconstrictors, and further eNOS uncoupling [41]. A major cause of eNOS uncoupling involves deficiency of the NOS cofactor tetrahydrobiopterin (BH₄) due to its decreased synthesis and/or increased oxidation [42]. eNOS-derived superoxide has been implicated in exaggerated blood cell–endothelial cell interaction in the cerebral microcirculation of SCD mice [6] through mechanisms possibly involving BH₄ deficiency. Elevated inactive eNOS monomers have also been reported in the lungs of transgenic SCD mice, and may be associated with pulmonary hypertension [7]. Loss of the active dimeric form of eNOS and predominance of inactive monomeric eNOS may explain compensatory upregulation of eNOS protein expression, yet decreased NO bioavailability, described in a transgenic SCD mouse model [43]. Uncoupled eNOS has recently been demonstrated to be a source of ROS in the penis of aged [44] and hypercholesterolemic [29,31] animals. We now report decreased ratio of functional eNOS dimers/nonfunctional eNOS monomers in the penis of SCD transgenic mice vs. their hemi counterparts in parallel with increased oxidative stress, implying uncoupled eNOS as a source of ROS in the SCD penis. It is important to note that eNOS uncoupling plays a key role in both the loss of NO biosynthetic capacity, and the increase in endothelial oxidative stress, both contributing to vascular dysfunction in the SCD penis.

In isolated endothelial cells [45], aorta of hypertensive mice [46,47], and penis of hypercholesterolemic mice [29], ROS produced by NADPH oxidase initiate eNOS uncoupling. In order to determine whether NADPH oxidase is an upstream activator of eNOS uncoupling in the penis of sickle mice, we treated these mice with apocynin. Apocynin is a specific inhibitor of NADPH oxidase activation, which both prevents assembly of cytosolic units with the membrane complex and negatively regulates NADPH oxidase subunits expression [29,32]. We present data showing that inhibition of NADPH oxidase by apocynin decreased protein expressions of p47^{phox} and gp91^{phox} subunits, as expected, and decreased lipid peroxidation in the penis of SCD mice. Apocynin treatment

also slightly reduced eNOS uncoupling, implying that ROS generated by NADPH oxidase are partially, although not solely, responsible for eNOS uncoupling. These findings invoke a novel role for NADPH oxidase as a major source of ROS in the SCD penis. The lack of reversal of eNOS coupling by NADPH oxidase inhibition indicates that other ROS sources may also contribute to eNOS uncoupling in the SCD penis. The role for xanthine oxidase in ROS production has been demonstrated in the lungs of SCD mice [5]. Furthermore, other mechanisms for eNOS uncoupling, which are unrelated to oxidation of the enzyme or its cofactor BH₄ [48], may also operate in the SCD penis. They may include decreased BH₄ synthesis or decreased availability of the NOS substrate L-arginine. Suboptimal endothelial arginine levels have been reported in the lungs of SCD mice, reflecting increased arginase activity [7] and elevated asymmetric dimethylarginine (ADMA) [49]. Future studies are needed to explore the exact mechanism of eNOS uncoupling in the SCD penis.

The pro-oxidant environment in the SCD penis may be due not only to high production of ROS, but also to a reduction in antioxidant systems. SOD, which converts superoxide to hydrogen peroxide, exists in three isoforms: cytoplasmic copper-zinc (SOD1), mitochondrial manganese (SOD2), and extracellular copper-zinc (SOD3). Catalase, mainly expressed in peroxisomes, catalyzes the decomposition of hydrogen peroxide to water and molecular oxygen. GPx reduces hydrogen peroxide to water, and lipid peroxides to their corresponding alcohols [50]. Eight different isoforms of GPx have been discovered. GPx1 is the ubiquitous isoform expressed in the cytosol and mitochondria in most cells, including the endothelium. Recent studies indicated a critical role of GPx1 in preserving NO bioavailability and protecting the cardiovascular system against oxidative stress [51,52]. The few studies that have examined antioxidant enzymes in patients or mice with SCD have yielded contradictory results with respect to levels of SOD and catalase [11,13]. Our studies demonstrate unaltered protein expressions of SOD1, SOD2, and catalase, and decreased protein expression of GPx1 in the SCD mouse penis. Low levels of GPx1 may promote susceptibility to oxidative stress by allowing accumulation of harmful oxidants, thus further contributing to eNOS uncoupling, increased oxidative stress, and conceivably endothelial dysfunction.

A limitation of this study is that we only investigated the molecular changes in ROS sources, selective enzymatic antioxidants, and oxidative stress, in the SCD mouse penis. In vivo erectile studies will be performed in the future to evaluate whether the priapic phenotype is decreased by NADPH oxidase inhibition, which would corroborate our findings.

Conclusions

SCD increases protein expressions of NADPH oxidase subunits, induces eNOS uncoupling, and decreases antioxidant GPx1 in the SCD penis, resulting in increased oxidative stress. Apocynin decreases oxidative stress through inhibition of NADPH oxidase upregulation, implying a major role for NADPH oxidase as a ROS source in the SCD penis. The findings suggest that targeting a specific ROS-generating source in the penis may offer a potential treatment for SCD-related priapism.

Acknowledgments

This work was supported by NIH/NIDDK grant RO1DK067223 to ALB.

References

1. Wood KC, Granger DN. Sick cell disease: Role of reactive oxygen and nitrogen metabolites. Clin Exp Pharmacol Physiol. 2007; 34:926–32. [PubMed: 17645642]

2. Wood KC, Hsu LL, Gladwin MT. Sick cell disease vasculopathy: A state of nitric oxide resistance. *Free Radic Biol Med*. 2008; 44:1506–28. [PubMed: 18261470]
3. Elahi MM, Kong YX, Matata BM. Oxidative stress as a mediator of cardiovascular disease. *Oxid Med Cell Longev*. 2009; 2:259–69. [PubMed: 20716913]
4. Wood KC, Hebbel RP, Granger DN. Endothelial cell NADPH oxidase mediates the cerebral microvascular dysfunction in sickle cell transgenic mice. *FASEB J*. 2005; 19:989–91. [PubMed: 15923406]
5. Pritchard KA Jr, Ou J, Ou Z, Shi Y, Franciosi JP, Signorino P, Kaul S, Ackland-Berglund C, Witte K, Holzhauser S, Mohandas N, Guice KS, Oldham KT, Hillery CA. Hypoxia-induced acute lung injury in murine models of sickle cell disease. *Am J Physiol Lung Cell Mol Physiol*. 2004; 286:L705–14. [PubMed: 12972407]
6. Wood KC, Hebbel RP, Lefer DJ, Granger DN. Critical role of endothelial cell-derived nitric oxide synthase in sickle cell disease-induced microvascular dysfunction. *Free Radic Biol Med*. 2006; 40:1443–53. [PubMed: 16631534]
7. Hsu LL, Champion HC, Campbell-Lee SA, Bivalacqua TJ, Mancini EA, Diwan BA, Schimel DM, Cochard AE, Wang X, Schechter AN, Noguchi CT, Gladwin MT. Hemolysis in sickle cell mice causes pulmonary hypertension due to global impairment in nitric oxide bioavailability. *Blood*. 2007; 109:3088–98. [PubMed: 17158223]
8. Hasanato RM. Zinc and antioxidant vitamin deficiency in patients with severe sickle cell anemia. *Ann Saudi Med*. 2006; 26:17–21. [PubMed: 16521870]
9. Prasad AS. Zinc deficiency in patients with sickle cell disease. *Am J Clin Nutr*. 2002; 75:181–2. [PubMed: 11815307]
10. Varma RN, Mankad VN, Phelps DD, Jenkins LD, Suskind RM. Depressed erythrocyte glutathione reductase activity in sickle cell disease. *Am J Clin Nutr*. 1983; 38:884–7. [PubMed: 6650447]
11. Natta CL, Chen LC, Chow CK. Selenium and glutathione peroxidase levels in sickle cell anemia. *Acta Haematol*. 1990; 83:130–2. [PubMed: 2109451]
12. Dasgupta T, Fabry ME, Kaul DK. Antisickling property of fetal hemoglobin enhances nitric oxide bioavailability and ameliorates organ oxidative stress in transgenic-knockout sickle mice. *Am J Physiol Regul Integr Comp Physiol*. 2010; 298:R394–402. [PubMed: 20007516]
13. Schacter L, Warth JA, Gordon EM, Prasad A, Klein BL. Altered amount and activity of superoxide dismutase in sickle cell anemia. *FASEB J*. 1988; 2:237–43. [PubMed: 3350236]
14. Das SK, Nair RC. Superoxide dismutase, glutathione peroxidase, catalase and lipid peroxidation of normal and sickled erythrocytes. *Br J Haematol*. 1980; 44:87–92. [PubMed: 7378296]
15. Montague DK, Jarow J, Broderick GA, Dmochowski RR, Heaton JP, Lue TF, Nehra A, Sharlip ID. Members of the Erectile Dysfunction Guideline Update Panel; Americal Urological Association. Guideline on the management of priapism. *J Urol*. 2003; 170:1318–24. [PubMed: 14501756]
16. Burnett AL, Bivalacqua TJ. Priapism: Current principles and practice. *Urol Clin North Am*. 2007; 34:631–42. [PubMed: 17983902]
17. Burnett AL, Bivalacqua TJ. Priapism: New concepts in medical and surgical management. *Urol Clin North Am*. 2011; 38:185–94. [PubMed: 21621085]
18. Wen J, Jiang X, Dai Y, Zhang Y, Tang Y, Sun H, Mi T, Phatarpekar PV, Kellems RE, Blackburn MR, Xia Y. Increased adenosine contributes to penile fibrosis, a dangerous feature of priapism, via A2B adenosine receptor signaling. *FASEB J*. 2010; 24:740–9. [PubMed: 19858092]
19. Bivalacqua TJ, Musicki B, Hsu LL, Gladwin MT, Burnett AL, Champion HC. Establishment of a transgenic sickle-cell mouse model to study the pathophysiology of priapism. *J Sex Med*. 2009; 6:2494–504. [PubMed: 19523035]
20. Broderick GA, Kadioglu A, Bivalacqua TJ, Ghanem H, Nehra A, Shamloul R. Priapism: Pathogenesis, epidemiology, and management. *J Sex Med*. 2010; 7:476–500. [PubMed: 20092449]
21. Burnett AL, Bivalacqua TJ, Champion HC, Musicki B. Long-term oral phosphodiesterase 5 inhibitor therapy alleviates recurrent priapism. *Urology*. 2006; 67:1043–8. [PubMed: 16698365]
22. Burnett AL, Bivalacqua TJ, Champion HC, Musicki B. Feasibility of the use of phosphodiesterase type 5 inhibitors in a pharmacologic prevention program for recurrent priapism. *J Sex Med*. 2006; 3:1077–84. [PubMed: 17100941]

23. Champion HC, Bivalacqua TJ, Takimoto E, Kass DA, Burnett AL. Phosphodiesterase-5A dysregulation in penile erectile tissue is a mechanism of priapism. *Proc Natl Acad Sci U S A*. 2005; 102:1661–6. [PubMed: 15668387]
24. Mi T, Abbasi S, Zhang H, Uray K, Chunn JL, Xia LW, Molina JG, Weisbrodt NW, Kellems RE, Blackburn MR, Xia Y. Excess adenosine in murine penile erectile tissues contributes to priapism via A2B adenosine receptor signaling. *J Clin Invest*. 2008; 118:1491–501. [PubMed: 18340377]
25. Kanika ND, Tar M, Tong Y, Kuppam DS, Melman A, Davies KP. The mechanism of opiorphin-induced experimental priapism in rats involves activation of the polyamine synthetic pathway. *Am J Physiol Cell Physiol*. 2009; 297:C916–27. [PubMed: 19657052]
26. Musicki B, Champion HC, Hsu LL, Bivalacqua TJ, Burnett AL. Post-translational inactivation of endothelial nitric oxide synthase in the transgenic sickle cell mouse penis. *J Sex Med*. 2011; 8:419–26. [PubMed: 21143412]
27. Kanika ND, Melman A, Davies KP. Experimental priapism is associated with increased oxidative stress and activation of protein degradation pathways in corporal tissue. *Int J Impot Res*. 2010; 22:363–73. [PubMed: 21085184]
28. Paszty C, Brion CM, Mancini E, Witkowska HE, Stevens ME, Mohandas N, Rubin EM. Transgenic knockout mice with exclusively human sickle hemoglobin and sickle cell disease. *Science*. 1997; 278:876–8. [PubMed: 9346488]
29. Musicki B, Liu T, Lagoda GA, Strong TD, Sezen SF, Johnson JM, Burnett AL. Hypercholesterolemia-induced erectile dysfunction: Endothelial nitric oxide synthase (eNOS) uncoupling in the mouse penis by NAD(P)H oxidase. *J Sex Med*. 2010; 7:3023–32. [PubMed: 20626609]
30. Hurt KJ, Musicki B, Palese MA, Crone JC, Becker RE, Moriaruty JL, Snyder SH, Burnett AL. Akt-dependent phosphorylation of endothelial nitric oxide synthase mediated penile erection. *Proc Natl Acad Sci U S A*. 2002; 99:4061–6. [PubMed: 11904450]
31. Musicki B, Liu T, Strong T, Jin L, Laughlin MH, Turk JR, Burnett AL. Low-fat diet and exercise preserve eNOS regulation and endothelial function in the penis of early atherosclerotic pigs: A molecular analysis. *J Sex Med*. 2008; 5:552–61. [PubMed: 18194184]
32. Wedgwood S, Lakshminrusimha S, Farrow KN, Czech L, Gugino SF, Soares F, Russell JA, Steinhorn RH. Apocynin improves oxygenation and increases eNOS in persistent pulmonary hypertension of the newborn. *Am J Physiol Lung Cell Mol Physiol*. 2012; 302:L616–26. [PubMed: 22198908]
33. Yang Y, Sharma R, Sharma A, Awasthi S, Awasthi YC. Lipid peroxidation and cell cycle signaling: 4-hydroxynonenal, a key molecule in stress mediated signaling. *Acta Biochim Pol*. 2003; 50:319–36. [PubMed: 12833161]
34. Muneer A, Celik S, Ralph DJ, Minhas S. The investigation of putative agents, using an in vitro model, to prevent cavernosal smooth muscle dysfunction during low-flow priapism. *BJU Int*. 2008; 102:988–92. [PubMed: 18565173]
35. Montezano AC, Burger D, Ceravolo GS, Yusuf H, Montero M, Touyz RM. Novel Nox homologues in the vasculature: Focusing on Nox4 and Nox5. *Clin Sci (Lond)*. 2011; 120:131–41. [PubMed: 21039341]
36. Shukla N, Hotston M, Persad R, Angelini GD, Jeremy JY. The administration of folic acid improves erectile function and reduces intracavernosal oxidative stress in the diabetic rabbit. *BJU Int*. 2009; 103:98–103. [PubMed: 18778356]
37. Jin HR, Kim WJ, Song JS, Piao S, Choi MJ, Tumurbaatar M, Shin SH, Yin GN, Koh GY, Ryu JK, Suh JK. Intracavernous delivery of a designed angiopoietin-1 variant rescues erectile function by enhancing endothelial regeneration in the streptozotocin-induced diabetic mouse. *Diabetes*. 2011; 60:969–80. [PubMed: 21270241]
38. Jin L, Lagoda G, Leite R, Webb RC, Burnett AL. NADPH oxidase activation: A mechanism of hypertension-associated erectile dysfunction. *J Sex Med*. 2008; 5:544–51. [PubMed: 18208505]
39. Claudino MA, Franco-Penteado CF, Priviero FB, Camargo EA, Teixeira SA, Muscará MN, De Nucci G, Zanesco A, Antunes E. Upregulation of gp91phox subunit of NAD(P)H oxidase contributes to erectile dysfunction caused by long-term nitric oxide inhibition in rats: Reversion by regular physical training. *Urology*. 2010; 75:961–7. [PubMed: 19962729]

40. Kietadisorn R, Juni RP, Moens AL. Tackling endothelial dysfunction by modulating NOS-uncoupling: New insights in pathogenesis and therapeutic possibilities. *Am J Physiol Endocrinol Metab.* 2012; 302:E481–95. [PubMed: 22167522]
41. Chen W, Druhan LJ, Chen CA, Hemann C, Chen YR, Berka V, Tsai AL, Zweier JL. Peroxynitrite induces destruction of the tetrahydrobiopterin and heme in endothelial nitric oxide synthase: Transition from reversible to irreversible enzyme inhibition. *Biochemistry.* 2010; 49:3129–37. [PubMed: 20184376]
42. Alp NJ, Channon KM. Regulation of endothelial nitric oxide synthase by tetrahydrobiopterin in vascular disease. *Arterioscler Thromb Vasc Biol.* 2004; 24:413–20. [PubMed: 14656731]
43. Kaul DK, Liu XD, Chang HY, Nagel RL, Fabry ME. Effect of fetal hemoglobin on microvascular regulation in sickle transgenic-knockout mice. *J Clin Invest.* 2004; 114:1136–45. [PubMed: 15489961]
44. Johnson JM, Bivalacqua TJ, Lagoda GA, Burnett AL, Musicki B. eNOS-uncoupling in age-related erectile dysfunction. *Int J Impot Res.* 2011; 23:43–8. [PubMed: 21289638]
45. Zhang M, Song P, Xu J, Zou MH. Activation of NAD(P)H oxidases by thromboxane A2 receptor uncouples endothelial nitric oxide synthase. *Arterioscler Thromb Vasc Biol.* 2011; 31:125–32. [PubMed: 20947827]
46. Landmesser U, Dikalov S, Price SR, McCann L, Fukai T, Holland SM, Mitch WE, Harrison DG. Oxidation of tetrahydrobiopterin leads to uncoupling of endothelial cell nitric oxide synthase in hypertension. *J Clin Invest.* 2003; 111:1201–9. [PubMed: 12697739]
47. Dikalova AE, Gongora MC, Harrison DG, Lambeth JD, Dikalov S, Griendling KK. Upregulation of Nox1 in vascular smooth muscle leads to impaired endothelium-dependent relaxation via eNOS uncoupling. *Am J Physiol Heart Circ Physiol.* 2010; 299:H673–9. [PubMed: 20639222]
48. Crabtree MJ, Channon KM. Synthesis and recycling of tetrahydrobiopterin in endothelial function and vascular disease. *Nitric Oxide.* 2011; 25:81–8. [PubMed: 21550412]
49. Schnog JB, Teerlink T, van der Dijs FP, Duits AJ, Muskiet FA. Plasma levels of asymmetric dimethylarginine (ADMA), an endogenous nitric oxide synthase inhibitor, are elevated in sickle cell disease. *Ann Hematol.* 2005; 84:282–6. [PubMed: 15599544]
50. Loscalzo J. Antioxidant enzyme deficiencies and vascular disease. *Expert Rev Endocrinol Metab.* 2010; 5:15–8. [PubMed: 20190873]
51. Blankenberg S, Rupprecht HJ, Bickel C, Torzewski M, Hafner G, Tiret L, Smieja M, Cambien F, Meyer J, Lackner KJ. AtheroGene Investigators. Glutathione peroxidase 1 activity and cardiovascular events in patients with coronary artery disease. *N Engl J Med.* 2003; 349:1605–13. [PubMed: 14573732]
52. Dayal S, Brown KL, Weydert CJ, Oberley LW, Arning E, Bottiglieri T, Faraci FM, Lentz SR. Deficiency of glutathione peroxidase-1 sensitizes hyperhomocysteinemic mice to endothelial dysfunction. *Arterioscler Thromb Vasc Biol.* 2002; 22:1996–2002. [PubMed: 12482825]

Figure 1.

Effect of SCD on protein expressions of GPx1, SOD1, SOD2, and catalase in the penis. Left panels are representative Western immunoblots. Right panels represent quantitative analysis of GPx1, SOD1, SOD2, and catalase in penes of hemi and sickle mice. Each bar represents the mean $\pm$ SEM of 5–7 mice. * $P < 0.05$ vs. hemi

Figure 2.

Effect of SCD and apocynin treatment on protein expressions of NADPH oxidase subunits in the penis. Upper panels are representative Western immunoblots of the NADPH oxidase subunits in penes of hemi, hemi treated with apocynin, sickle, and sickle mice treated with apocynin. Lower panels represent quantitative analysis of NADPH oxidase subunits p67^{phox}, p47^{phox}, and gp91^{phox} in penes in the same treatment groups. Each bar represents the mean $\pm$ SEM of 5–7 mice. * P < 0.05 vs. hemi; ** P < 0.05 vs. sickle + vehicle; Vehic = Vehicle; Apoc = Apocynin

Figure 3.

Effect of SCD and apocynin treatment on eNOS uncoupling in the penis. Upper panel is a representative Western immunoblot of the dimers/monomers in penes of hemi, hemi treated with apocynin, sickle, and sickle mice treated with apocynin. Lower panel represents quantitative analysis of eNOS dimer/monomer ratio in penes in the same treatment groups. Each bar represents the mean $\pm$ SEM of 6–7 mice. * $P < 0.05$ vs. hemi; Vehic = Vehicle; Apoc = Apocynin

Figure 4.

Effect of SCD and apocynin treatment on 4-HNE-modified proteins in the penis. Upper panel is a representative Western immunoblot of 4-HNE in penes of hemi, hemi treated with apocynin, sickle, and sickle mice treated with apocynin. Lower panel is a quantitative analysis of 4-HNE-modified proteins in penes in the same treatment groups. The analysis of 4-HNE is a densitometric composite of all proteins in each lane. Each bar represents the mean $\pm$ SEM of 5–7 mice. * $P < 0.05$ vs. hemi; ** $P < 0.05$ vs. sickle + vehicle; Vehic = Vehicle; Apoc = Apocynin