Nitric Oxide in the Penis: Scientific Discoveries and Clinical Applications

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Disclosure Statement: In accordance with the ACCME policy on relevant financial disclosure, I disclose financial relationships with the following entities: American Medical Systems, Auxilium Inc, Coloplast, Endo Pharmaceuticals, National Institutes of Health, Pfizer Inc, Reflexonic LLC
Acknowledgments

**Urology**
- Biljana Musicki, Trinity Bivalacqua, Thomas Chang

**Neuroscience**
- Solomon Snyder, David Bredt, K. Joseph Hurt

**Cardiology**
- Charles Lowenstein, Hunter Champion, David Kass

**Hematology**
- James Casella, Samuel Charache, Lewis Hsu

**Reproductive Biology**
- Barry Zirkin, Terry Brown
THE POTENCY PILL
Yes, VIAGRA works! And the craze says a lot about men, women and sex.
JUST SAY NO

MOLECULE OF THE YEAR
Overview

- Multiple actions of nitric oxide in the penis
  - Physiologic penile erection
  - Penile vascular health
  - Homeostasis and interaction with other signaling molecules

- Therapeutic relevance
  - Erectile dysfunction
  - Recurrent ischemic priapism
  - Penile fibrosis
HUMAN PENILE ERECTION

Psychogenic Mechanism
- Erotic Stimuli
  - Visual
  - Auditory
  - Olfactory
  - Gustatory
  - Memory
  - Imagination

Reflexogenic Mechanism
- Tactile Stimuli

Limbic System
- Sympathetic N.S.
- Parasympathetic N.S.

Thoracolumbar Outflow
(T10 – L4)

Hypogastric nerve

Pelvic Plexus

Sacral Outflow
(S2 – S4)

Cavernous nerve

Pelvic nerve

Pudendal nerve
Functional anatomy of the erect penis

Based on the description of the vein-occlusive mechanism proposed by Lue.²

Cavernosal artery

Locular spaces

Draining venules

Helicoid arteries

Tunica albuginea

Draining venules

Noncompressed draining venules

Flaccid state

Blood-filled locular spaces

Contracted helicoid artery

Constricted trabecular smooth muscle

Dilated helicoid artery

Relaxed trabecular smooth muscle

Erection

Tunica albuginea

Constricted trabecular smooth muscle
A New Observation…

Leads to a New Question…

and to a New Discovery
Suddenly, Nitric Oxide is in the News!
Nitric Oxide: A Factor in Erectile Tissue Relaxation

- Direct application of nitric oxide relaxes isolated muscle strips from the corpus cavernosum similar to nerve stimulation.
- In vitro tissue relaxation effects are blocked by inhibitors of nitric oxide synthesis.
- “Neurotransmitter identity doubt” – proof needed that nitric oxide is released from neurons.

Nitric oxide (NO) is a cytotoxic agent of macrophages, a messenger molecule of neurons, and a vasodilator produced by endothelial cells. NO synthase, the synthetic enzyme for NO, was localized to rat penile neurons innervating the corpora cavernosa and to neuronal plexuses in the adventitial layer of penile arteries. Small doses of NO synthase inhibitors abolished electrophysiologically induced penile erections. These results establish NO as a physiologic mediator of erectile function.
Nitric Oxide Synthase is Localized to the Autonomic Innervation of the Human Penis

Control of Trabecular Smooth Muscle Contractility
Clinical Therapeutics: Target Sites

A New Observation…

Leads to a New Question…

and to a New Discovery
Nitric Oxide Synthase Knockout Mice

- Developed to investigate the involvement of nitric oxide in various biological functions\(^1\)
- Focus on nNOS, eNOS, double NOS mutant mice in sexual physiology research
- Challenge: these mice preserve copulatory ability!\(^2\)
  - Why, if this regulatory pathway is essential?
  - Do alternative mechanisms permit sexual function?

1. Huang PL. Semin Perinatol 24, 87-90, 2004
Neuronal NOS Knockouts

- eNOS-dependent mechanisms are retained\(^1\)
- Lack nNOS \(\alpha\) (which encodes exon 2) but preserve nNOS splice variants\(^2\)
- nNOS \(\beta\) is expressed and is physiologically relevant\(^3\)

Endothelial NOS Knockouts

- Display supra-normal erections to electrophysiologic stimulation\(^1\)
- Display attenuated erections to pharmacologic stimulation\(^2\)

Akt-dependent phosphorylation of endothelial nitric-oxide synthase mediates penile erection

K. Joseph Hurt, Bulijana Musicki, Michael A. Palese, Julie K. Crone, Robyn E. Becker, John, L. Morriarity, Solomon H. Snyder, and Arthur L. Burnett
Molecular Mechanisms that Underlie Penile Erection

1. STIMULUS
   - PSYCHOGENIC
   - REFLEXOGENIC

   NEURONAL DEPOLARIZATION

   Ca^{2+}

   CaM - nNOS

   NO

2. INITIATION BY NEURAL NO

3. PROLONGATION BY ENDOTHELIAL NO

   RELAXATION AND SHEAR STRESS

   CONTINUED RELAXATION

   PI3K

   Akt

   eNOS

   P
Regulation of eNOS is the Key to Penile Vascular Health
Towards Better Penile Health: Hypothesis

Vasoactive Therapy

↓

Erectile Tissue Relaxation

↓

Intrapenile Blood Flow Stimulation

↓

Activation of Endothelial NOS

↓

Penile Vascular Repair and Restoration
A New Observation…

Leads to a New Question…

and to a New Discovery
Erection Excess in eNOS-/- Mice

PDE5 Dysregulation In Penile Erectile Tissue: Mechanism Of Priapism

- NOS3 -/- mice had enhanced erectile response to CNS.
- eNOS gene transfer to the NOS3-/- mouse penis resulted in neurogenic-mediated erectile responses similar to WT mice via an elevation of PDE5A expression/activity.

Thus, properly regulated PDE5 function under physiologically relevant NO signaling preserves normal erection physiology.

Therefore, if penile PDE5 expression is dysregulated priapism occurs.

Biochemical Activity Measurements in Sickle Cell Mouse Penes

SS-/- mice show a priapic phenotype, and this is associated with reductions of both NOS and PDE5A activities.
A Reversed Nitrate Tolerance Mechanism?

[Diagram showing the mechanism of NO, PDGF/Ang II, Ca^{2+}, PGI_2, cGMP, cAMP, GMP/AMP, cGMP-induced activation of PDE5, upregulation of PDE1A, induction of PDE1C, relaxation, nitro-tolerance, and proliferation.]
Mechanism of Priapism
Interim Summary

Hypothesis

Sickle Cell Disease

PDE5 Inhibitor Therapy

eNOS

Endothelial-NO

PDE5

Priapism

Restore Normal Penile Vascular Homeostasis

Penile Vasculature
Effect of Chronic PDE5 Inhibitor Therapy on Erectile Responses in Wild Type and Transgenic Sickle Cell Mice

3 Week Sildenafil Treatment
Randomized Controlled Trial of Sildenafil for Preventing Recurrent Ischemic Priapism in Sickle Cell Disease

Arthur L. Burnett, MD, MBA, Uzoma A. Anele, MD, Irene N. Trueheart, RN, John J. Strouse, MD, PhD, James F. Casella, MD

Background: Successful preventive therapy for ischemic priapism, a disorder of penile erection with major physical and psychologic consequences, is limited. We conducted a randomized, double-blind, placebo-controlled clinical trial to assess the efficacy and safety of sildenafil by a systematic dosing protocol to prevent recurrent ischemic priapism associated with sickle cell disease.

Methods: Thirteen patients with sickle cell disease reporting priapism recurrences at least twice weekly were randomized to receive sildenafil 50 mg or placebo daily, unassociated with sleep or sexual activity, for 8 weeks, followed by open-label use of this sildenafil regimen for an additional 8 weeks.

Results: Priapism frequency reduction by 50% did not differ between sildenafil and placebo groups by intention-to-treat or per protocol analyses (P = 1.0). However, during open-label assessment, 5 of 8 patients (62.5%) by intention-to-treat analysis and 2 of 3 patients (66.7%) by per protocol analysis met this primary efficacy outcome. No significant differences were found between study groups in rates of adverse effects, although major priapism episodes were decreased 4-fold in patients monitored “on-treatment.”

Conclusions: Sildenafil use by systematic dosing may offer a strategy to prevent recurrent ischemic priapism in patients with sickle cell disease.

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Keywords: Erection; Erectile dysfunction; Nitric oxide; Phosphodiesterase type 5
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Role of NO and cGMP in Erections

Sexual stimulation

Corpus cavernosum

Erection

Smooth muscle relaxation

NO=nitric oxide

NANC=nonadrenergic, noncholinergic neurons

GTP=guanosine triphosphate

cGMP=cyclic guanosine monophosphate

PDE5=phosphodiesterase type 5.

cGMP=cyclic guanosine monophosphate. GTP=guanosine triphosphate. NANC=nonadrenergic, noncholinergic neurons. NO=nitric oxide. PDE5=phosphodiesterase type 5.
Sustained phosphorylation of nNOS-S1412 after electrical stimulation of rat MPG.

Intracavernous pressure increases with intrapenile injection of forskolin in wild-type but not nNOS-/-mice or wild-type after L-NAME pretreatment.

PNAS 109(2012), 16624-9
Integrative Model of nNOS and eNOS Regulation of Initiation and Maintenance of Erectile Function
## NOS Roles in Penile Biology

<table>
<thead>
<tr>
<th>Function</th>
<th>Isoform</th>
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<tbody>
<tr>
<td>Erection Mediation</td>
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</tr>
<tr>
<td>Initiator</td>
<td>nNOS α/β, eNOS</td>
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<tr>
<td>Facilitator</td>
<td>P-nNOS, P-eNOS</td>
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<td>Erectile Tissue Preservation</td>
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<tr>
<td>Vasculoprotector</td>
<td>P-eNOS</td>
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<tr>
<td>Anti-fibrosis agent</td>
<td>iNOS</td>
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<td>Homeostasis</td>
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<tr>
<td>Biochemical Modulator</td>
<td>eNOS</td>
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“Gladly I think of the days when all my members were limber, all except one.

Those days are certainly gone, now all my members are stiff, all except one.”

- Goethe