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Laser Oximetry: A Novel Noninvasive Method to Determine Changes in Penile Hemodynamics in an Anesthetized Rabbit Model

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ABSTRACT: This study was designed to determine the utility and validity of laser oximetry in measuring changes in penile hemodynamics. Anesthetized male New Zealand White Rabbits were divided into 2 groups, and penile hemodynamics were assessed by either laser oximetry (oxyhemoglobin, deoxyhemoglobin concentration, and oxygen saturation) or intracavernosal pressure (ICP) monitoring during penile erection induced by pelvic nerve stimulation (PNS) or intracavernosal administration of phentolamine, nitroprusside, papaverine, or sildenafil. PNS caused significant frequency-dependent increases in penile ICP. PNS also caused significant increases in penile tissue oxyhemoglobin concentrations and tissue oxygen saturation in a frequency-dependent manner. The changes in oxyhemoglobin concentrations and oxygen saturation correlated with frequency-dependent increases in ICP. Intracavernosal vasoactive drug administration produced significant increases in ICP, tissue oxyhemoglobin concentration, oxygen saturation, and duration of response as a function of increasing drug concentration. Laser oximetry permits reproducible and valid assessment of changes in penile hemodynamics comparable to conventional ICP measurements. Thus, we consider laser oximetry a reliable technique in evaluating penile hemodynamics. Its sensitivity in detecting small changes in oxyhemoglobin concentration and its noninvasive nature make it advantageous over invasive methods such as ICP monitoring and laser Doppler flowmetry.

Key words: Penile erection, oxyhemoglobin, near-infrared spectroscopy, intracavernosal pressure, pelvic nerve stimulation, tissue oxygen saturation.

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Sexual stimulation in the male results in a dramatic increase in penile cavernosal arterial inflow, intracavernosal pressure (ICP), and cavernosal oxygen saturation. These hemodynamic changes have been documented by a number of techniques, including laser Doppler flowmetry, direct measurement of ICP, and tissue oxygen tension (Azadzoi et al, 1995; Cahill et al, 1998; Mills et al, 1998). Each of these methodologies requires placement of a cannula or probe into the corpora cavernosa. The invasive nature of these techniques may produce alterations in the normal physiological responses because of direct tissue damage. In addition, laser Doppler flowmetry is highly operator-dependent. Laser oximetry, a continuous wave optical spectrometer operating in the near-infrared spectrum, provides a new noninvasive tool to evaluate changes in tissue perfusion. Laser oximetry permits the determination of oxyhemoglobin and deoxyhemoglobin concentrations in a targeted area of soft tissue. The utility and validity of this technique in measuring changes in penile tissue hemodynamics after pelvic nerve stimulation (PNS) or intracavernosal administration of vasoactive agents, however, have not been established.

In the mid-1980s, in vivo optical monitoring of skeletal muscle during hypoxia in animals using an experimental spectroscopy apparatus was reported (Piantadosi and Jöbsis-VanderVliet, 1985; Piantadosi et al, 1986). These studies measured tissue oxygenation variables using near-infrared spectrum (700–900 nm) spectroscopy and correlated tissue oxygenation with blood flow (Jöbsis, 1977). Near-infrared light penetrates biological tissue, allowing transmission spectrometry to be performed through dense tissue structures (Wyatt et al, 1986). Technological advances in oximetry have reduced the size and cost of the oximeter, thereby increasing its potential applicability (Scoggin et al, 1977). Smaller oximeter probe designs are available for measurements at bodily extremities such as the ear, finger, toe, or nose in humans (Rebuck et al, 1983). However, these miniature probes transmit light through a tissue bed to a sensor positioned on the other side, limiting their usefulness for larger organs or tissue

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structures. More recently, the development of fiber optic array probes consisting of multiple light sources and 1 detector coupled to a continuous wave optical spectrometer makes it possible to penetrate a targeted volume of tissue (>500 mm^3) to an average depth of 5–8 mm beneath the skin surface. The detector fiber is positioned in the same plane as the source fibers and collects scattered light. By directly measuring scattering and absorption of near-infrared light, oxyhemoglobin and deoxyhemoglobin concentrations can be accurately determined. Laser oximetry with multiple source probes has been used to measure blood flow in the fetal brain (Sutterlin et al, 1999; Jacques et al, 2000). Therefore, laser oximetry may be a useful tool for the assessment of local penile tissue hemodynamics and oxygenation in a noninvasive and continuous fashion.

This study was designed to determine the validity and usefulness of laser oximetry in measuring penile hemodynamics by comparing oxyhemoglobin concentration and oxygen saturation with ICP measurements, as a parameter of erectile function. We used laser oximetry to monitor penile tissue hemodynamics before, during, and after penile erection, in response to PNS and intracavernosal administration of vasoactive agents.

Materials and Methods

Drugs

Papaverine hydrochloride was purchased from Research Biochemicals International (Natick, Mass). Phentolamine mesylate was obtained from Ciba-Geigy Corp (Summit, NJ). Sildenafil citrate was a generous gift from Dr Farid Saad (Jenapharm GmbH, Jena, Germany). All other drugs and reagents were obtained from Sigma Chemical Co (St Louis, Mo) or other commercially available sources.

Animals

All studies were approved by the Institutional Animal Care and Use Committee of the Boston University School of Medicine. Male New Zealand White Rabbits (3.5–4.0 kg) were sedated with intramuscular ketamine (35 mg/kg) and xylazine (5 mg/kg) and placed in the supine position. Anesthesia was maintained as needed with intravenous sodium pentobarbital (50 mg/mL). A 3-cm midline neck incision was fashioned to access the carotid artery. A 20-gauge angiocatheter was inserted into the carotid artery and connected to a PT300 pressure transducer (Grass Instruments/Astromed Inc, Warwick, RI) to continuously monitor systemic blood pressure. Body temperature was maintained with an electric heat pad.

Pelvic Nerve Stimulation

A 4-cm lower midline abdominal incision was fashioned to expose the pelvic nerve, which can be identified on the posterolateral aspect of the rectum. Bladder contents were aspirated through the bladder wall with an 18-gauge needle and a 50-mL syringe. Under direct vision, a bipolar platinum wire electrode was hooked onto the pelvic nerve without cutting the nerve. Unilateral PNS was accomplished with a Grass S9 stimulator set at normal polarity and repeat mode to generate a 30-second train of square waves with a 10-V pulse amplitude, a 0.8-ms pulse width, and varying frequencies (2–32 Hz). The interval between stimulations was 10–15 minutes to prevent nerve exhaustion. Physiological parameters were measured at baseline and then during and after PNS or intracavernosal administration of vasoactive agents.

ICP Monitoring

Under sterile conditions, the skin overlying the penis was incised, and the corpora cavernosa was exposed at the root of the penis. A 23-gauge needle filled with 50 U/mL heparin solution and connected to PE-50 tubing was inserted into the left corpus cavernosum for pressure recording. All pressure measurements were recorded by means of Grass PT-300 pressure transducers connected to PI-1-ACDC signal conditioner modules and a Grass 7400 physiological recorder (Astro-Med).

Drug Administration

A 30-gauge needle filled with 50 U/mL heparin solution and connected to PE-10 tubing was inserted into the right corpus cavernosum for intracavernosal drug administration. Each drug was dissolved in normal saline and administered in a final volume of 0.1 mL. The change in ICP was monitored with each dose, and all pressure responses were allowed to return to baseline before the subsequent dose. Changes in ICP are expressed as a fractional change in ICP in relation to systemic arterial pressure (SAP) (ie, ICP/SAP).

Laser Oximetry

We utilized a dual-channel oximeter (Model 96208, ISS Inc, Champaign, Ill). To maximize contact with the optical fibers, the skin around the penis was carefully shaved. The probe (2 cm in length) was positioned longitudinally over the penis such that the detector fiber was positioned just below the pubic arch. The probe assembly was secured in place by a metal stand. The area over the probe was covered with a black cloth to prevent any interference from natural or artificial ambient light sources.

Data Analysis

Oxyhemoglobin and oxygen saturation data are reported as the difference between the basal and the peak response values. ICPs were normalized to SAP and expressed as a ratio (ICP/SAP). The duration of response was defined as the time interval between the initial rise in oxyhemoglobin or ICP and the decay to baseline values. Data were expressed as the mean plus or minus the standard deviation. All comparisons were performed by *t* test relative to the lowest stimulation frequency or the lowest concentration of drug. Data were considered statistically significant when the *P* value was less than or equal to .05.

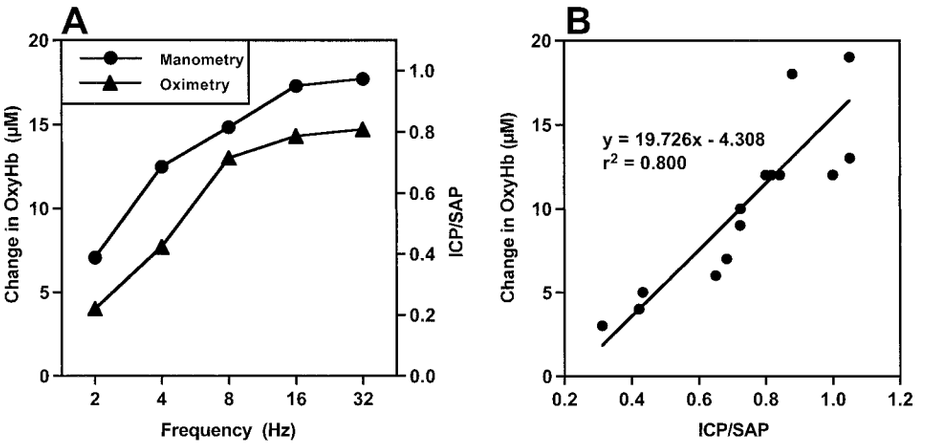

Figure 1. Changes in oxyhemoglobin concentration and intracavernosal pressure (ICP) in response to pelvic nerve stimulation (PNS) **(Panel A)** and correlation between oxyhemoglobin concentration and intracavernosal pressure/systemic arterial pressure (ICP/SAP) **(Panel B)**.

Results

Effects of PNS on Penile Hemodynamics

In one group of animals (n = 3), we determined changes in ICP in response to PNS at increasing frequencies (2, 4, 8, 16, and 32 Hz). The mean basal ICP/SAP value in the absence of any stimulation was 0.18 plus or minus 0.04. PNS elevated ICP in a frequency-dependent manner (Figure 1A). In a second group of animals (n = 3), we examined PNS-induced changes of tissue oxyhemoglobin concentration and oxygen saturation in the penis. Basal levels of oxyhemoglobin and oxygen saturation ranged between 28–35 μM and 40%–50%, respectively. PNS caused significant frequency-dependent increases in penile tissue oxyhemoglobin concentrations and tissue oxygen saturation with a concomitant decrease in deoxyhemoglobin concentration. The duration of recorded responses measured by the 2 techniques also increased with respect to increasing frequency of nerve stimulation (Table 1). The changes in ICP were positively correlated with the changes in oxyhemoglobin concentrations, determined by laser oximetry (r = .841) (Figure 1B).

Table 1. *Changes in hemodynamic parameters after PNS**

PNS Frequency (Hz)	Oximetry		Manometry	
	Change in O_2 Saturation (%)	Duration (min)	ICP/SAP	Duration (min)
2	1.8 ± 0.2	1.5 ± 0.1	0.39 ± 0.05	1.5 ± 0.3
4	3.3 ± 0.6	1.8 ± 0.2	0.69 ± 0.03†	1.7 ± 0.1
8	7.0 ± 2.0†	2.5 ± 0.0†	0.82 ± 0.08†	1.8 ± 0.5
16	7.7 ± 2.1†	2.7 ± 0.3†	0.95 ± 0.13†	1.8 ± 0.0
32	8.3 ± 1.8†	3.5 ± 0.3†	0.97 ± 0.10†	2.0 ± 0.1

* All values are mean ± SD. ICP indicates intracavernosal pressure; PNS, pelvic nerve stimulation; and SAP, systemic arterial pressure.
† $P < .05$, relative to 2-Hz response.

Effects of Vasodilator Agents on Penile Hemodynamics

We examined the changes of penile tissue oxyhemoglobin concentrations and oxygen saturation after intracavernous administration of 4 vasodilators. The changes in ICP were measured in separate animals, and their resultant parameters were compared with laser oximetry parameters (Figure 2; Table 2). Phentolamine (0.05, 0.15, 0.3, and 1.0 mg/kg; n = 3), sodium nitroprusside (1, 3, 10, and 30 μg/kg; n = 3), papaverine (0.1, 0.3, 0.6, and 1.0 mg/kg; n = 3), or sildenafil (3, 10, 30, 75, and 150 μg/kg; n = 3) caused significant increases in ICP in a dose-dependent manner. In parallel experiments using laser oximetry (n = 12), we observed a dose-dependent increase in tissue oxyhemoglobin concentrations and oxygen saturation. The durations of recorded responses were also observed to increase with respect to increasing doses of vasodilators. Among the vasodilators used in this study, sodium nitroprusside (30 μg/kg) caused the largest increase, elevating ICP up to that of SAP, and increased total hemoglobin concentration 26.3 plus or minus 2.2 μM. However, papaverine (1.0 mg/kg) and sildenafil (150 μg/kg) enhanced the response duration to a greater extent than sodium nitroprusside (Table 2). The changes in oxyhemoglobin and oxygen saturation produced in response to the vasodilators and measured by laser oximetry correlated well with those determined by ICP measurements (Figure 2).

Discussion

The purpose of this study was to determine the validity and usefulness of laser oximetry in measuring changes in penile hemodynamics as a parameter of erectile function. This study demonstrated that laser oximetry detected

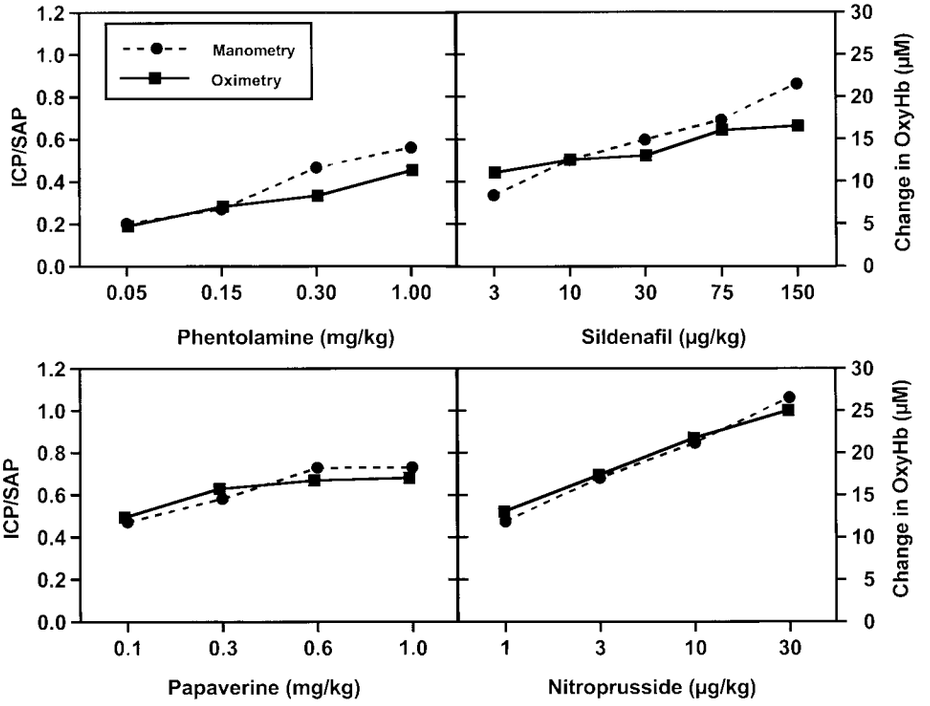

Figure 2. Changes in hemodynamic parameters in response to intracavernosal administration of vasoactive agents.

changes in oxyhemoglobin concentrations in response to PNS or pharmacologically induced penile erection. PNS caused a significant frequency-dependent increase in penile blood flow, as determined from changes in tissue oxyhemoglobin concentrations and tissue oxygen saturation. When changes in oxyhemoglobin concentrations were plotted as a function of frequency (Hz), they paralleled ICP measurements. This suggests that measurement of oxyhemoglobin concentrations by laser oximetry is a valid parameter to assess changes in penile hemodynamics.

The response duration for PNS-induced erection was similar regardless of frequency when measured by manometry, whereas the response duration increased with frequency when measured by oximetry. This distinction reveals a key difference in the 2 methodologies. The increase in oxyhemoglobin occurs rapidly after PNS because of the dilation of the resistance arteries that regulate blood flow into the penile cavernosal bodies. However, pressure does not develop within the penis until a later time when blood is trapped within the organ. This trapping of blood does not occur until the penis has expanded in girth and length sufficiently to engage the veno-occlusive mechanism, which is accomplished by the stretching and compression of subtunical venules. This temporal relationship between increasing oxygenation and pressure is corroborated by the simultaneous measurements of blood PO_2 and ICP that have been previously reported (Kim et al, 1993). In our animal model, maintenance of ICP is dependent on continuous stimulation of the pelvic nerve. Discontinuation of active PNS interrupts veno-occlusion and results in a rapid decrease in ICP. Since the period of nerve stimulation (30 seconds) did not change, the duration of the pressure response was also similar. However, partial penile tumescence may continue even in the absence of veno-occlusion. At higher frequencies of stimulation, this period of partial tumescence is prolonged. Thus, the mean changes in oxyhemoglobin concentrations reflect the acute hemodynamic response in penile tissue secondary to PNS, while the duration of response reflects hemodynamic engorgement or blood-retaining properties in penile tissue.

Different disease states have the potential to alter hematocrit and/or oxyhemoglobin concentrations. However, the hemodynamic events that must occur to attain full penile erection remain the same. Thus, the use of laser oximetry in evaluating the erectile response would be largely unaffected. The ability to sensitively detect changes in oxyhemoglobin and tissue oxygen saturation may be advantageous in studying erectile dysfunction associated with vascular insufficiency states that can occur with diseases such as atherosclerosis and diabetes.

To confirm that laser oximetry parameters determined in response to physiological stimulation (PNS) mimic changes in the well-documented, pharmacologically induced increase in ICP, we determined oxyhemoglobin

Table 2. *Changes in hemodynamic parameters after intracavernosal administration of 4 vasodilators**

	Oximetry		Manometry	
Drug Dose	Change in O_2 Saturation (%)	Duration (min)	ICP/SAP	Duration (min)
Phentolamine (mg/kg)				
0.05	4.7 ± 1.5	19.3 ± 5.0	0.20 ± 0.05	8.2 ± 0.7
0.15	6.7 ± 1.5	31.3 ± 4.6	0.27 ± 0.11	18.7 ± 2.4†
0.3	7.0 ± 1.0†	39.3 ± 7.5	0.47 ± 0.14	30.2 ± 6.9†
1.0	8.3 ± 1.1	75.0 ± 20.0	0.56 ± 0.49†	52.8 ± 3.7†
Nitroprusside (μg/kg)				
1	6.7 ± 1.5	26.0 ± 6.9	0.47 ± 0.13	13.4 ± 2.5
3	8.7 ± 2.3	50.0 ± 8.7†	0.68 ± 0.09	35.2 ± 8.9†
10	11.3 ± 3.1	72.0 ± 15.9†	0.85 ± 0.30	47.2 ± 6.6†
30	14.0 ± 2.0†	110.0 ± 13.3†	1.06 ± 0.18	90.0 ± 9.0†
Papaverine (mg/kg)				
0.1	5.7 ± 0.6	113.3 ± 11.5	0.47 ± 0.07	44.3 ± 16.7
0.3	7.7 ± 1.5	170.0 ± 17.3†	0.58 ± 0.19	50.5 ± 13.4
0.6	9.7 ± 2.1	186.7 ± 11.5†	0.72 ± 0.08	61.2 ± 2.53
1.0	9.7 ± 1.1†	200.0 ± 26.7	0.73 ± 0.07	118.4 ± 33.8
Sildenafil (μg/kg)				
3	4.0 ± 0.0	83.3 ± 11.5	0.34 ± 0.09	17.2 ± 0.9
10	5.5 ± 0.7	113.3 ± 11.6	0.50 ± 0.05	37.8 ± 18.2
30	8.0 ± 2.8	193.3 ± 11.6	0.60 ± 0.04	50.6 ± 15.2
75	10.0 ± 1.2†	220.0 ± 28.3†	0.69 ± 0.09†	56.3 ± 3.9†
150	12.0 ± 2.0†	290.0 ± 33.3†	0.86 ± 0.11†	126.8 ± 30.4

* All values are mean ± SD. ICP indicates intracavernosal pressure; SAP, systemic arterial pressure.
† $P < .05$, relative to lowest dose of each drug.

concentrations and ICP after intracavernosal injection of phentolamine, papaverine, nitroprusside, or sildenafil. Oxyhemoglobin concentrations increased in a dose-dependent fashion in response to all vasodilators tested. These measurements were paralleled by similar increases in ICP. These observations indicate that laser oximetry is a useful and valid tool for the evaluation of changes in penile hemodynamics in response to pharmacological manipulation.

Phentolamine, nitroprusside, and papaverine are well-known vasodilators. It is interesting that sildenafil, developed as an oral medication, caused a strong and long-acting vasodilatory effect after intracavernosal administration, without PNS. The accepted mechanism of sildenafil action is the inhibition of the enzyme phosphodiesterase type 5 in penile tissue. Since the inhibition of phosphodiesterase type 5 prevents cyclic guanosine monophosphate (cGMP) hydrolysis but cannot directly stimulate cGMP production, it was thought that nitric oxide generated by sexual stimulation was indispensable for sildenafil to be effective. In view of this observation, sildenafil administered intracavernosally may act through another mechanism (McAuley et al, 2001).

An intrinsic advantage of laser oximetry is its noninvasive nature. Furthermore, since one of the early events in penile erection is increased arterial inflow, the measurement of penile tissue oxyhemoglobin concentrations is an excellent parameter in assessing cavernosal response to physiological and pharmacological stimuli. Recently, a different technology based on near-infrared spectrophotometry has been utilized in the clinical setting to evaluate erectile function (Burnett et al, 2000). Changes in penile blood volume were correlated with penile rigidity measurements in patients with erectile dysfunction and healthy volunteer subjects (Burnett et al, 2000). These observations lend support to the data presented in this communication. In addition, we have shown that laser oximetry permits assessment of female genital engorgement in response to pharmacological and physiological stimuli (Min et al, 2000). This technique has proven useful in the female animal model, where sexual stimulation induced by PNS produces genital engorgement instead of veno-occlusion (rigidity).

In conclusion, laser oximetry is a reproducible and reliable technique for the evaluation of penile hemodynamics. Its sensitivity in detecting small changes in oxyhemoglobin concentration and its noninvasive nature make it advantageous over other conventional methods such as ICP monitoring and laser Doppler flowmetry.

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