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MODULATION OF PENILE BLOOD FLOW THROUGH VASOACTIVE AGENTS

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Abstract

The erectile mechanism is a sequential cascade of events, involving many chemical messengers, perhaps the most important one being nitric oxide (NO). NO is a putative neurotransmitter involved in the non-adrenergic non-cholinergic (NANC) system and is synthesized by the enzyme nitric oxide synthase (NOS). NOS has been localized in peripheral autonomic nerves innervating vascular and non-vascular smooth muscles in many organ systems.

The introduction of intracavernous injection (ICI) of vasoactive agents in 1981 was an effective means to restore erectile function for men with erectile dysfunction (ED). Recent evidence supports the theory that increased blood flow can induce the release of NO from vascular endothelium, suggesting that ICI may have positive benefits for the host.

Our study looked at (1) a new delivery system for intracavernous injection and (2) the effect of modulation of penile flow on the regulation of NOS content and activity. A canine model assessed the effectiveness of a subcutaneous drug delivery as an alternate means to ICI. Additionally, a paraplegic rat model was developed to assess the effects of chronic ICI of papaverine on the expression of NOS in the penile tissue.

Our first objective, testing a new subcutaneous drug delivery system, yielded no data due to technical difficulties. The experiment involving the rat model, our results demonstrated that ICI of papaverine significantly increased the number of NOS fibers within the penile shaft, indicating that an increase in the flow of blood within the penis can alter levels of NOS within penile tissue. This result may in part explain the observation seen in patients, whereby after 1 year of ICI, spontaneous erections return and ICI therapy may be discontinued.

Résumé

Le mécanisme érectile est une série d'évènements en cascade impliquant plusieurs messagers chimiques dont peut-être le plus important: l'oxyde nitrique (NO). NO est un neuro-transmetteur impliqué dans le système non-adrénergique, non-cholinergique (NANC) et est synthétisé par l'enzyme nitrique oxyde synthase (NOS). NOS est localisé dans les nerfs périphériques autonomes, innervant les muscles lisses vasculaires et non-vasculaires de plusieurs organes.

L'apparition de l'utilisation chronique d'agent vasoactif par injection intra-caverneuse (ICI), en 1981, a été une innovation qui a permis de restaurer la fonction érectile d'une grande majorité d'homme avec une dysfonction érectile (ED). Malheureusement, ICI, est associé à des complications locales tel que cicatrices, fibrose et des problèmes techniques lors de l'injection. Également, la peur de l'injection est un facteur important qui fait chuter le taux de réussite entre 36 et 53%. Des études récentes suggèrent qu'un flux sanguin élevé peut induire la production d'oxyde nitrique dans l'endothélium vasculaire suggérant que ICI peut avoir un ou des effets positifs à long terme chez le patient.

Notre étude portait sur (1) un nouveau système d'injection intra-caverneux, (2) l'effet de la fluctuation du niveau de flux sanguin dans le pénis sur la régulation de la quantité de NOS et de son activité.

Un modèle canin a été utilisé afin d'étudier l'efficacité d'un système sous-cutané d'injection de drogue comme alternative à ICI. Parallèlement, un modèle de rat paraplégique a été développé afin d'étudier les effets chroniques de ICI d'un agent vaso-actif (papaverine) sur l'expression de NOS dans le tissus pénien.

Nos résultats démontrent que ICI chronique de papaverine provoque une hausse de la quantité de NOS contenu dans les fibres du corps pénien ce qui indique qu'une augmentation du flux sanguin au niveau du pénis peut altérer les niveaux de NOS dans le tissus pénien. Ce résultat expliquerait l'observation notée chez des patients qui après une année de ICI, ont été capable d'avoir une érection satisfaisante.

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ATP:	Adenosine Triphosphate
BP:	Blood Pressure
cAMP:	Cyclic Adenosine Monophosphate
cGMP:	Cyclic Guanosine Monophosphate
cNOS:	Constitutive Nitric Oxide Synthase
DICC:	Dynamic Infusion Cavernosography and Cavernosometry
ED:	Erectile Dysfunction
EDRF:	Endothelium Derived Relaxing Factor
EFS:	Electric Field Stimulation
GTP:	Guanosine Triphosphate
ICI:	Intracavernous Injection
ICP:	Intracavernous Pressure
IIEF:	International Index of Erectile Function
iNOS:	Inducible Nitric Oxide Synthase
L-NAME:	NG-Nitro-L-Arginine-Methyl-Ester
L-NMMA:	L-N-Monomethyl-Arginine
L-NOARG:	L-N-Nitroarginine
mRNA	Messenger Ribonucleic Acid
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NANC:	Nonadrenergic Noncholinergic
NNLA:	N-nitro L-arginine
NO:	Nitric Oxide
NOS:	Nitric Oxide Synthase
NPT:	Nocturnal Penile Tumescence
NTs:	Neurotransmitters
PDE5:	Phosphodiesterase Type 5
PGE_1:	Prostaglandin E_1
REM:	Rapid Eye Movement
SD:	Standard Deviation
SEM :	Standard Error of the Mean
SCI:	Spinal Cord Injured
T11-L2:	Thoracic 11-Lumbar 2

Chapter 1 - Introduction

1.1-Background

Despite its taboo nature, sex is an essential and vital element of human life. Humans are the only species that engage in sex for reasons other than procreation. The Starr-Weiner report released in 1981 found that 93% of American people over 60 were still sexually active.[1] In 1988, Bretschneider and McCoy[2] surveyed a group of healthy men over age 80 and found that 29% of the males surveyed were having intercourse at least once a week. Despite this, there exists a large percentage of males who are no longer able to engage in sexual intercourse. It is estimated that 55% of all men over the age of 75 are impotent.[3] This high number is rather startling in view of the fact that 20-30 million men in North America alone suffer with erectile dysfunction, yet only a small minority have consulted a physician in order to obtain treatment for their problem.[4] Erectile dysfunction results in more than 400,000 outpatient visits and 30,000 hospital admissions annually in North America.[5] As the median age of the population rises, this number is expected to increase even further. Erectile dysfunction is not, and should not be viewed as a normal consequence of aging. The decrease in sexual activity that results with increasing age is mainly due to erectile dysfunction whose origin is related to co-morbid conditions that are associated with the aging process.[6] Diabetes, hypercholesterolemia, atherosclerosis and a history of smoking, play an essential role in the development of erectile dysfunction.[7]

Society has come a long way since the nineteenth century when scientists thought that erectile dysfunction could be attributed to a single cause.[8] Today, it is understood that there are many factors that contribute to the development of ED. These factors can be broken down into many categories: vascular (arterial and venous blood supply), neurogenic, hormonal and psychogenic. It is essential that all of these components are functioning within a normal range because obtaining and maintaining an erection is a multifactorial event. Therefore, abnormalities in any one of these systems may result in erectile dysfunction. Our understanding of the anatomy, physiology and neuronal components involved in the erectile process has lead to the development of treatment

approaches for the majority of those afflicted. The introduction of intracavernous vasoactive agents in 1981 revolutionized the treatment of this condition, providing a minimally invasive, effective means to restore erectile function for the majority of impotent men.

1.2-Anatomy

The penile erectile tissue is composed of two corporal bodies that are positioned dorsally and are known as the corpora cavernosa. The distal end of these two cylindrical structures are joined but as they travel proximally the two corpora cavernosa diverge to form the left and right crus. The crura are joined to the ipsilateral ischiopubic ramus of the pelvic girdle. The septum between the two corpora cavernosa remains incomplete, allowing for the exchange of blood between the two sides. However, some species, such as the dog, possess a complete septum, thus preventing the two bodies from communicating with one another. The third corporal body is located ventrally and is known as the corpus spongiosum. The corpus spongiosum houses the penile urethra and enlarges distally to form the glans penis.

A thick layer of fibrous tissue surrounds each of the three corpora. This fibrous layer, known as the tunica albuginea separates the three corporal bodies from one another. The tunical covering is much thicker around the corpora cavernosa, than around the corpus spongiosum and is complete except for those areas in which nerves and/or arteries penetrate. The tunica is absent around the glans penis. The tunica is composed of bundles of collagen and elastin fibers. These fibers are woven in a criss-cross pattern, which maintains the structural integrity of the penis and can accommodate a high degree of elongation and expansion. A single layer of fascia known as Buck's fascia covers all three corporal bodies and lies superficial to the tunica albuginea.

The erectile tissue within the corpus cavernosum is composed of a meshwork of interconnected sinusoidal spaces. These spaces are separated by trabeculae, which are composed of bundles of smooth muscles in a framework of fibroblasts, collagen and elastic fibers[9]. In contrast, the erectile tissue of the corpus spongiosum contains significantly less smooth muscle and the sinusoidal spaces are larger.

1.2.1-Arterial Blood Supply

The arterial blood supply to the penis originates from the paired internal pudendal arteries, which themselves are branches of the hypogastric arteries.[10] The pudendal artery divides and gives off several branches. Two of these branches go to the corpus spongiosum as the urethral artery and the bulbar artery and continue distally to the glans.

The remaining two branches of the internal pudendal artery form the dorsal penile artery and the deep penile artery. The dorsal artery travels distally along the corpus cavernosum between the tunica albuginea and Buck's fascia. The deep penile artery also travels distally; however, it penetrates the crus and runs within the corpus cavernosum. Once inside the corpus cavernosum it is referred to as the deep (central) artery. This artery supplies vessels to the trabeculae and helical arteries. These helical arteries end as short arteries that enter directly into the sinusoidal spaces of the corpus cavernosum.

1.2.2-Venous Drainage

X-ray findings have demonstrated the existence of 3 separate intercommunicating venous drainage systems of the penis. The superficial dorsal penile vein is responsible for the superficial venous drainage of the penis. Located between Colle's and Buck's fascia, the superficial dorsal penile vein is responsible for venous outflow from the prepuce and penile skin. The superficial dorsal penile vein empties into the saphenous vein, which empties into the femoral vein. Deep venous drainage is accomplished via the deep dorsal penile vein. This vein is located in a groove of the corpora cavernosa and lies between the tunica albuginea and Buck's fascia. It is responsible for draining the glans penis, the corpus spongiosum and to a lesser extent the corpora cavernosa. Finally there are the venae profundae, which originate directly from the cavernous bodies.[9]

1.2.3-Innervation

The penis is innervated by two sets of nerves: autonomic (parasympathetic and sympathetic) and somatic (sensory and motor). Autonomic neurons originating from the spinal cord as well as from peripheral ganglia merge to form the cavernous nerves. The parasympathetic nerve fibers arise from neurons whose cell bodies are located in the sacral portion of the spinal cord. The sympathetic nerves originate from the eleventh thoracic to the second lumbar vertebrae (T11-L2). The sensory pathway begins at the

sensory receptors in the skin of the penis, glans, and urethra and within the corpus cavernosum. The nerve fibers from these various receptors converge to form bundles of the dorsal nerve of the penis. These bundles join other nerves and become the internal pudendal nerve. The center of the somatomotor penile innervation is Onuf's nucleus.[8] These nerves travel with the sacral nerves to the pudendal nerve to innervate the bulbocavernous and ishiocavernous muscles.

1.3-Physiology

1.3.1-Hemodynamics

Penile erection and detumescence are primarily hemodynamic events that involve the arterial and venous systems. Whether the contribution made by the arterial system is of greater importance than that of the venous system remain questionable. In the 19th century, venous occlusion was thought to be the main factor in achieving and maintaining an erection. In the middle of the twentieth century investigators emphasized the importance of high arterial flow. Experiments by such investigators as Dorr and Brody,[18] Shirai et. al.[19] and Wagner et. al.[20] demonstrated the importance of arterial flow in producing and maintaining an erection. However, the landmark research by Lue et al.[21] and Lue and Tanagho[22] has demonstrated that the venous system does play an important role in the ability to achieve and sustain an erection through passive compression and limitation of outflow after a period of rapid arterial filling of the corpora.

1.3.1.1-Arterial dynamics

In the flaccid state sympathetic influences from the autonomic nervous system are dominant. The smooth muscle within the cavernous tissue is in a state of contraction, thus reducing the size of the sinusoidal spaces. As a result, a minimal amount of blood flows through the sinusoids, just enough to provide nutrients to the cavernosal tissue. In 1980 Wagner and Uhrenholdt[23] estimated this flow to be 2.5-8 ml/min/100g of tissue. During sexual stimulation the parasympathetic and NANC systems become dominant and this results in relaxation of the smooth muscle within the corporal bodies. As a result of this relaxed state the trabeculae musculature increases in compliance. This allows the sinusoidal spaces to expand and in turn allows for a greater flow of arterial blood to enter.

The blood is retained in the expanding sinusoidal spaces resulting in expansion and elongation of the penis. When a full erection is reached the pressure within the cavernosum is 10-20 mm Hg below the systolic blood pressure.

1.3.1.2-Venous dynamics

When the penis is in the flaccid state the sinusoids and arterioles are constricted, allowing a small amount of blood to perfuse the cavernous tissues. However, the venules, which carry blood to the emissary veins, are free flowing. During erection the arterioles dilate and the sinusoidal spaces distend, thereby compressing the venules between the walls of the sinusoids and the relatively rigid tunica albuginea. This cascade of events effectively reduces the venous outflow, trapping the blood within the confines of the penis, allowing the penis to remain erect.

1.3.2-Neurophysiology

The parasympathetic, NANC and sympathetic neurons merge to form the cavernous nerves, which enter the three corporal bodies to mediate the neurovascular events that take place during erection and detumescence. Stimulation of the pelvic plexus induces erection. Conversely, stimulation of the hypogastric nerve results in detumescence. This illustrates that the sacral parasympathetic input is responsible in part for erection, and the thoracolumbar sympathetic input regulates detumescence to some degree. The somatic neurons are responsible for sensation of the penis and the contraction of the bulbocavernosus and ischiocavernosus muscles. The internal pudendal nerve is formed from receptor nerve fibers. Activation of these sensory receptors sends messages such as pain, temperature and touch. Eventually these inputs are relayed to the thalamus and sensory cortex. These nerves innervate the bulbocavernosus and ischiocavernosus muscles.[8] Contraction of these muscles increases the intracavernous pressure (ICP), which is the rigid erection phase.

1.4-Phases of Penile Erection

The state of erection can be divided into 7 phases, including the normal or flaccid state. During the flaccid state there is minimal blood flowing to the sinusoidal spaces. The arterial flow velocity is approximately 15cm/s or less. The following briefly outlines the

unique features and/or events of each of the 7 phases that are involved in the erectile process as described by Dr. Tom F. Lue.[8]

1. Filling: Blood flow is highest during this phase and the penis elongates with no change to the intracavernous pressure (ICP). Flow velocity reaches 30cm/s with an increase in flow in the internal pudendal and cavernous arteries.
2. Tumescence: There's an increase in the intracavernous pressure and a decrease in the arterial blood flow. The penis is now fully elongated and blood enters only during the systolic phase.
3. Full erection: ICP is steady and is approximately 90% of the systolic blood pressure.
4. Rigid erection: Due to contractions from the ischiocavernosus muscle, the ICP rises above the systolic blood pressure, resulting in a rigid erection. During this phase there is no inflow of blood.
5. Initial detumescence: This phase is marked by a small transient increase in the ICP.
6. Slow detumescence: A slow decline in the ICP is seen resulting in opening of the once compressed venous channels and a decrease in arterial flow.
7. Fast detumescence: A rapid decline in ICP and the venous system is fully restored.

In summary, penile erection is the result of intracorporeal smooth muscle relaxation, increase in compliance of the sinusoidal spaces, and increase arterial flow and venous resistance.

1.5-Neuropharmacology

1.5.1-Neurotransmitters

The neurotransmitter and its neuroeffects are not well understood in the human model. Acetylcholine, as recognized for ganglionic transmission (by nicotinic receptors) and vascular smooth muscle relaxations (by muscarinic receptors), has been thought to be the primary neurotransmitter responsible for erection. However, intracavernous or intravenous injections of atropine (reversible competitive blockade of acetylcholine), failed to abolish erections induced in animals by electric field stimulation (EFS)[24,25] and in men by erotic stimuli.[23] In addition intracavernous injections of acetylcholine failed to produce a full erection. These results suggested that neurotransmitters other than acetylcholine might be involved.[11]

Investigations were then directed to catecholamines (epinephrine and norepinephrine) because the vasculature and smooth muscle within the corpus cavernosum is rich in adrenergic nerves.[10] Experiments illustrated that the net effect of adrenergic stimulation resulted in detumescence rather than erection. It was thus concluded that the mechanism of erection could not be explained by the classical cholinergic and adrenergic mechanisms.[10]

Research was then focused on non-adrenergic non-cholinergic neurotransmitters. The stimulation of the cavernous nerve led to a significant degree of corporal smooth muscle relaxation.[5] The discovery and recognition of endothelium-derived relaxing factor (EDRF) as a major participant in the physiology of vascular smooth muscle relaxation promoted new insights into the mechanism of penile erection.[10] Subsequently EDRF has been recognized as nitric oxide and numerous researchers have suggested that nitric oxide is the most important mediator of penile smooth muscle relaxation.[11]

1.6-Nitric Oxide

Nitric oxide is synthesized from endogenous L-arginine by the enzyme nitric oxide synthase, which catalyzes the oxidation of L-arginine into citrulline and nitric oxide. Once synthesized, nitric oxide is released locally from nerve fibers and the endothelium. Nitric oxide is an unstable gaseous compound with a short half-life and its production can be competitively blocked by L-arginine analogues such as L-N-monomethyl-arginine (L-NMMA) and L-N-nitroaginine (L-NOARG) which substitutes for L-arginine.

In vitro animal and human studies have shown that EFS of isolated cavernous smooth muscle strips evoked relaxation of the cavernosal smooth muscle. Using NOS inhibitors this state of relaxation can be blocked. This strongly supports the hypothesis that NO is a NANC neurotransmitter involved in the relaxation of cavernosal smooth muscle.

Nitric oxide causes penile smooth muscle relaxation that mimics the effects of EFS. This reaction occurred in the presence of both guanethidine and atropine, a cathecolamine depletor and a muscarinic receptor antagonist, respectively.[11] Another experiment showed that relaxation due to NO could be further increased by treating the

strips of smooth muscle with a cGMP phosphodiesterase inhibitor.[10] These observations lend additional support to the hypothesis that stimulation of NANC neurons within the corpus cavernosum leads to an increase in the endogenous formation of NO in either neurons, smooth muscles or endothelial cells.[10]

Endothelial cells produce a constitutive form of nitric oxide synthase known as cNOS as well as an inducible form of nitric oxide synthase known as iNOS. cNOS is sensitive to Ca^{2+} whereas iNOS is Ca^{2+} insensitive. Once released and after penetrating the cell, nitric oxide activates guanylate cyclase which increases intracellular levels of cyclic guanosine monophosphate (cGMP) by converting guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP). In turn cGMP signals protein kinases which activate ion channels and induce smooth muscle relaxation. Another mechanism also exists which involves activation of adenylate cyclase to convert adenosine triphosphate (ATP) into cyclic adenosine monophosphate (cAMP).[11] In the early 1990s an experiment was setup to evaluate the role of cAMP, cGMP, the endothelium and the NANC nervous system on penile erection.[11] Based on the results of their experiments, they derived the following conclusions:

The action of acetylcholine is totally dependent on the integrity of the sinusoidal endothelium. When the endothelium is destroyed the activity of acetylcholine is non-existent.

An erectile response resulting in a change in intracavernous pressure (ICP) between 60-85 cm H_2O can be elicited by neurostimulation in the absence of an endothelium, indicating that the release of neurotransmitters responsible for penile erection is only partially endothelium dependent and that the nerve endings or smooth muscles may also release neurotransmitters.

After injecting cGMP and cAMP it was concluded that cGMP is a more important secondary messenger for cavernous smooth muscle relaxation. This is due to the fact that direct injection of cAMP produced a poor erectile response. It resulted in a change in ICP between 0-30 cm H_2O. Whereas injection of cGMP produced a good erectile response, resulting in an ICP change between 54-90 cm H_2O. Also, the inability of N-ethylmaleimide (adenylate cyclase inhibitor), to block the erectile response to

neurostimulation led investigators to conclude that the cAMP system plays a minor role in cavernous smooth muscle relaxation.

1.6.1-Hemodynamic Forces and Nitric Oxide

Nitric oxide is released from endothelial cells during exposure to laminar blood flow. The hemodynamic forces resulting from the flow of blood include two components; (1) shear stress, which is the tangential frictional force produced when blood flows over the endothelial surface, and (2) pressure stretch, a force that acts perpendicular to the vascular wall.[12] Hydrodynamic factors such as flow have been shown to stimulate nitric oxide release in a variety of *in vitro* systems. Experiments using the femoral artery and the left circumflex artery from mongrel dogs have shown endothelium-dependent vasodilatation in response to variations in blood flow. It was this observation that lead to the theory that endothelial cells release vasodilator mediators in response to an augmented blood flow. Initial experiments showed that as the flow rate increased there was an increase in the level of prostacyclin from cultured endothelial cells and from endothelium of perfused arteries. However, using indomethacin which is a cyclooxygenase inhibitor, the formation of prostacyclin was inhibited and no longer being released. Despite this inhibition the flow-induced vasodilatation was still present. It was concluded that the mediator that was being released from the endothelium was not prostacyclin. [13]

Signal transduction by external mechanical forces such as shear stress may occur via the cell membranes, by cell surface receptors, by activation of specific ion channels or by changes in the cell cytoskeleton. A potassium-selective, shear stress-activated ion channel has been described in bovine aortic endothelial cells.[14] Recently, activation of this channel linked to a pertussis toxin-sensitive G protein, with subsequent increased NO and cGMP formation, has been demonstrated when endothelial cells were exposed to laminar flow.[15] Myatt et al.[16] tested the hypothesis that altering shear stress (changes in flow and viscosity) over endothelial cells will stimulate nitric oxide release and action in the human fetal placental vasculature *in vitro*. Their study showed that when N-nitro-L-arginine (NNLA), a competitive substrate inhibitor of NOS, was used there was a statistically significant increase in perfusion pressure at a high rate of flow (10 ml/min.), but not at a low rate of flow (1 ml/min). The greater effect of NNLA at a flow rate of 10ml/min. compared with that of 1 ml/min. indirectly suggests there is greater release of

NO at the higher flow rate and hence flow is a stimulus to NO release. This evidence suggests that reduced flow generates less nitric oxide and hence increased resistance. Therefore, shear stress acting on the endothelium is responsible for flow-induced nitric oxide release. These results have provided the foundation for one of the experiments that will be described in this thesis.

1.6.2-Oxygen Tension and Nitric Oxide

Another factor involved in nitric oxide synthesis and release is oxygen tension. During erection the penis acts like a large reservoir accumulating blood and increasing in pressure. In order to successfully achieve this, the arteries must dilate and the smooth muscle within the trabeculae must relax. This relaxation is induced by the release of nitric oxide from nerves and the endothelium. The synthesis of nitric oxide requires L-arginine and oxygen as substrates. Hypoxic conditions in the corpus cavernosum of the rabbit demonstrated a significant reduction in the activity of nitric oxide synthase.[17] Thus, oxygen may be the rate-limiting factor for nitric oxide production in the penile corpus cavernosum. Physiologically low oxygen tension may play a role in the inability to achieve an erection by inhibiting the activity of nitric oxide synthase, which ultimately results in the inability to have corporal smooth muscle relaxation.

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Chapter 2 - Erection & Erectile Dysfunction

2.1-Erection

There are three main types of erection that the male can experience: sleep erection, psychogenic erection and reflex erection. The data coming from evaluating patients with anatomically well defined lesions of the central nervous system has provided important new information.

2.1.1-Sleep Erection

This type of erection occurs in the vast majority of men during approximately 30% of the time they spend asleep, more specifically 80% of sleep erections occur during REM.[1] Sleep erections are also known as nocturnal penile tumescence (NPT), however sleep erection or NPT can occur during the daytime, so the name is somewhat misleading. It is not known whether sleep erections use both the parasympathetic and sympathetic pathways or only one of these paths.

2.1.2-Psychogenic Erection

This type of erection results from sexual thoughts and occurs in all young and old neurologically intact males. Clinical evidence has shown that psychogenic erections can use both the sympathetic and parasympathetic pathways.

2.1.3-Reflex Erection

This can be seen in men with spinal cord injuries at the level of T9 or higher. In these patients sexual thoughts have no influence on erection, however, an erection can occur if the penis is stimulated manually.[12] In some neurologically intact men mechanical stimulation in the absence of sexual thought can cause erection but not in the majority of men. However, sexual thought can cause an erection more easily if the penis is being stimulated mechanically. Stimulation of the parasympathetic pathway alone can mediate reflex erection. One of the effects of sexual thoughts is to release the reflex from its inhibition.

2.2-Etiology of Erectile Dysfunction

The erectile mechanism is a dynamic event that involves a wide variety of components, which range from the neurovascular system to the endocrine system including the penile tissue itself. Pathologies or abnormalities in any of these systems could result in ED.

2.2.1-Psychogenic Impotence

The brain sends facilitatory and inhibitory messages to the spinal erection centers, which either induce or inhibit the erectile process via the spinal nerves. The exact mechanism of psychogenic inhibition is not fully understood. It can be the result of direct inhibition from the brain to the spinal centers or increased peripheral catecholamine levels coming from sympathetic (stress related) pathway that in turn renders the smooth muscle of the cavernous tissue less responsive to neurotransmitters.

2.2.2-Neurogenic Impotence

This type of impotency can be caused by diseases or dysfunction that affect the brain, spinal cord, cavernous and pudendal nerves as well as receptors in the arterioles and cavernous smooth muscle. In patients with an upper spinal cord lesion, 95% are capable of obtaining an erection (reflexogenic), however, only 25% of patients with a lower spinal cord injury can attain an erection (psychogenic). This suggests that the sacral parasympathetic neurons are a more important erectile center. An injury to the cavernous or pudendal nerves is not uncommon during pelvic surgery. Although most still experience some degree of erectile dysfunction post-operatively, nerve-sparing techniques during radical surgery of the prostate and bladder have provided patients with a chance of remaining potent.

2.2.3-Hormonal Impotence

Androgens are essential for male sexual maturity and, clinically, androgens are thought to be linked to sexual activity through their action on increased libido. Therefore, in the adult, androgen deficiency results in a loss of sexual activity and a decrease in the frequency and magnitude of nocturnal erections. Hypogonadal patients, on testosterone replacement therapy had an increase in penile rigidity and total tumescence time.[2]

The relationship between NOS activity and androgen levels was documented by Tillman et al.[3] who showed a 51% decrease in NOS activity measured in the rat penis one week following castration. This report clearly documented, in a biological fashion, the androgen sensitivity of NOS. More recently, a study by Brock et al.[2] showed a 63% decrease in physiological response to EFS in animals 10 days after castration. However, if the castrated animals received testosterone at sufficiently high doses (10 mg/kg), their response to EFS was similar to the non-castrated group. Whether immediately following castration or 21 days post castration, administration of testosterone was successful in producing near normal EFS responses. (Immediate replacement of 10 mg testosterone: 65.1 ± 6.6 cm H_2O; 21 day post-op replacement of 10 mg testosterone: 64.2 ± 6.1 cm H_2O; Control: 70.4 ± 3.5 cm H_2O).

2.2.4- Arteriogenic Impotence

In order to achieve an erection, a substantial increase in blood flow is needed. Therefore, any injury to the aorta, hypogastric, pudendal or penile arteries may result in an impairment of the vascular-erectile mechanism. Arteriogenic impotence is frequently manifested by slowly developing, inadequate penile rigidity where the erection is insufficient for satisfactory sexual intercourse. Efforts to understand arteriogenic impotence have involved retrospective studies in man and prospective studies in animal models.[4] In man, it has been shown that obstruction of the inflow arterial supply to the corporal bodies by atherosclerotic lesions associated with atherosclerotic vascular disease is associated with impotence.[5]

Using the New Zealand White Rabbit, an animal model of atherosclerotic vascular disease was created[13] by using a Fogarty balloon to induce endothelial injury in the common and external iliac arteries. In addition the animals were fed a high fat diet that was composed of 1.6% cholesterol mixed with 4% peanut oil for eight weeks. This combination of balloon de-endothelialization and cholesterol diet allowed for the development of an atherosclerotic-induced vascular disease animal model. Twenty-one out of the twenty-six animals that were fed the modified high cholesterol diet survived the full eight weeks. Ten animals had severe atherosclerotic lesions, five had moderate atherosclerotic lesions and six had minimal atherosclerotic lesions. Sixteen of the twenty-

one animals had erectile impairment assessed by the intracavernosal administration of papaverine.

Group	Number of Animals	Erectile Dysfunction
Severe atherosclerotic lesions	10	10
Moderate atherosclerotic lesions	5	4
Minimal atherosclerotic lesions	6	2
Total	**21**	**16**

Figure 1. Degree of atherosclerosis and the number of animals displaying erectile dysfunction. (From Azadzoi KM, Goldstein I. Erectile dysfunction due to atherosclerotic vascular disease: the development of an animal model. *J Urol.* 1992; 142:1675.)

In summary, these studies showed that hypercholesterolemia and atherosclerotic occlusive disease of the iliac arteries causes erectile dysfunction in the rabbit. It appeared that the ED of these animals was based in part, on the hemodynamic alterations within the iliac-hypogastric arteries. However, in this animal model there is the possibility that hypercholesterolemia - induced alterations in the corpus cavernosum may have played a role in the development of impotence.[4]

2.2.5- Venogenic Impotence

Dysfunction of the corporeal veno-occlusive mechanism is a major cause of impotency.[6,7] Quantitative and qualitative assessment of corporeal veno-occlusive dysfunction can be accurately obtained by cavernosometry and cavernosography performed after intracavernous vasoactive stimulation.[7,8] Venous occlusion, or the compression of the venules between the sinusoids and the tunica, is a fundamental process in the erectile mechanism. Consequently, excessive cavernous vein outflow can prevent penile rigidity. This increased venous outflow can be affected by numerous factors related to the tunica albuginea, the compliant trabeculae (normal collagen to smooth muscle ratio), the intact neuromuscular function and the endothelium.[9]

Tanagho et al. [10] designed a study to outline the anatomic basis of the venous occlusion that takes place during penile erection. Using a canine model they made corrosion casts of the penis. Papaverine was injected into one corpus cavernosum while

saline was injected into the contralateral corpus. Batson's solution was injected and once hardened the animal was sacrificed. The casts were then examined using a dissecting microscope and then scanning electron microscopy. The venular pathways were observed arising from the corporeal sinusoids, thus placing them in the unique position to be compressed against the tunica albuginea.[10]

2.2.6-Erectile tissue dysfunction

Gross changes of the penile erectile tissue are known to occur in individuals with Peyronie's disease, trauma, diabetes and priapism. [11] Patients with severe arterial disease have alterations to the erectile tissue. The smooth muscle cells show irregular contour, loss of basal lamina and fragmentation. The cytoplasm is devoid of contractile myofilaments and the endothelium is significantly altered.[11] These findings occur in patients with arterial insufficiency from various causes, such as diabetes mellitus, atherosclerosis, hypertension and old age.

Abnormal endothelium can lead to a decrease in the synthesis and release of NO resulting in impairment of erection. In addition any disturbance in the normal composition of the erectile tissue can lead to an alteration in compliance of the sinusoidal spaces (such as fibrosis and loss of penile smooth muscle). This in turn can result in inadequate perfusion of blood within the sinusoids which is detrimental to the erectile process.

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Chapter 3 - Diagnosis and Diagnostic Tools

3.1-Measuring Penile Blood Flow

In 1985 Lue et. al.[1] introduced the use of duplex ultrasonography as a non-invasive means to measure blood flow deep within the penis. Originally papaverine was injected into the penis of the patient and measurements were taken after 1, 3 and 5 minutes. Subsequently, with the popularity PGE_1 the protocol changed and by 1991 patients were receiving PGE_1 as the most common form of vasoactive stimulant. In addition they were asked to induce self-stimulation and the scan was done at 5, 15 and 30 minutes. B-mode images (visualization of the diameter of the cavernous arteries in the proximal penile shaft) and Doppler spectra were obtained with a 5 MHz linear array transducer. The first images were transverse, in order to evaluate the corporal bodies and rule out any calcification. Longitudinal images were taken to measure the inner diameter of the cavernous arteries. The Doppler angle correction cursor was adjusted to match the correct axis of flow. This provided information regarding flow velocities. This technique is very useful for assessing alterations in penile blood flow.

3.2-Measuring Tumescence and Rigidity

Historically, it has been difficult to distinguish between ED of an organic etiology versus ED of a psychogenic etiology. Many investigators consider nocturnal penile tumescence (NPT) and rigidity as the only objective test for the differential diagnosis of ED into these two main groups.[2] It had been shown that penile erections occurred regularly in men during the night. Eighty percent of NPT occurred during rapid eye movement (REM) sleep. It was not long until regular monitoring of erections during sleep in a sleep laboratory was performed in the evaluation of ED. Tumescence was measured with mercury strain gauges and rigidity was measured separately because a number of patients have normal tumescence but poor rigidity. Therefore penile rigidity was thought to be best monitored axially by a tonometer because axial rigidity was thought to correlate most accurately with the ability to achieve vaginal penetration.[3]

Clinicians believed that patients would be more at ease if they could be monitored in the privacy of their own homes rather than in a sleep clinic. In 1985, a portable home monitor was developed that could simultaneously monitor tumescence as well as rigidity. The information was stored on a computer chip that could later be downloaded and converted to a paper tracing and analyzed. The Rigiscan monitor has been successfully used to separate organic causes from psychogenic causes. The monitor has continued to be validated as a diagnostic tool that can verify biogenic impotence.[4] Recently, Rigiscan monitoring has been shown to be superior to a history and physical examination alone in diagnosing organic ED.[2] A study by Guay et al.[3] was conducted to see if the results from home penile tumescence and rigidity monitoring were comparable to those done in a sleep laboratory. Their results showed that monitoring of NPT and rigidity at home using the portable Rigiscan were comparable to those obtained in a laboratory. As well the home monitor is less cumbersome and less expensive than its laboratory counterpart.

3.3-Cavernosography

Cavernosography is a technique used by clinicians to provide a more detailed understanding of the corpus cavernosum venous drainage system from a radiological point of view.[5] Conventional cavernosography can assess the venous function of the penis by the isolated visualization of the cavernous bodies by means of a contrast material. Over the past few years this technique has undergone decisive modifications and the newer technique is known as dynamic cavernosography or more accurately dynamic infusion cavernosography and cavernosometry (DICC). Through the use of dynamic cavernosography accurate flow rates during artificially induced erections can be determined as well as the pressure values of the cavernosal bodies. Porst et al.[5] carried out dynamic cavernosography on 140 patients to illustrate the accuracy and validity of this technique as a diagnostic tool in the identification of venous incompetency. Their results showed that dynamic cavernosography is a reliable diagnostic tool in determining ED of venous origin.

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Chapter 4 - Pharmacotherapy

4.1-Background

Although some authorities advocate that the choice of therapy for the treatment of erectile dysfunction is based on the results of pharmacocavernosometry and cavernosography (DICC), accompanied by assessing neurovascular risk factors as well as the clinical characteristics, in most situations, the ultimate form of treatment depends on the goals and expectations of the patient. Depending on the nature and etiology of the ED different modalities of treatment may be selected and the chance of success determined. Currently several forms of therapy exist that are available to treat ED; oral vasoactive agents, vasoactive injections, vacuum devices, penile implants, and vascular surgery.

In 1983 Brindley[19] demonstrated that phenoxybenzamine injected into the corpora cavernosa could produce a sustained erection. However, this agent had a slow onset and showed carcinogenic effects in laboratory animals[1]. In 1984 Virag et al.[4] documented the use of papaverine as both a diagnostic and therapeutic tool for impotent men. Papaverine alone requires a high dose and has been associated with liver toxicity, priapism and penile scar formation. Virag and Adaiken[21] reported on the use of prostaglandin E1 that like the other drugs had advantages and disadvantages. Although pain was reported in nearly 20% of men using PGE_1, a much improved safety profile for this drug was noted. This set the foundation for intracavernous injection of vasoactive agents as a viable treatment for impotency. Further experimenting with different combinations and doses continued for several years and today the optimal vasoactive agents for ICI are still under investigation.

Despite the success that was achieved with ICI, the "Holy Grail" of impotence therapy is a drug that can be administered orally and produces an erection of sufficient quality and duration for intercourse and is devoid of side effects.[3] Apomorphine has been reported to be effective in causing erection in animals when administered parenterally. Although apomorphine has poor bioavailability in oral form it has been shown to be absorbed through mucous membranes.[2] A study was designed to test primarily men with psychogenic ED with one of four protocols (sublingual liquid, 5 mg tablet, aqueous nasal

spray and 3 and 4 mg controlled absorption tablets).[3] Patients receiving the sublingual liquid complained of severe nausea, those taking the 5mg tablets and the nasal spray had no significant results and the side effects were significant. Finally, eight out of twelve men using the controlled tablets responded well and achieved an erection. Despite some success in this study, the majority of patients experienced significant side effects. In addition the sample population was constructed only with individuals suffering from psychogenic ED. Apomorphine was far from the ideal therapy of ED, however the search for this elusive treatment continues.

4.2-Sildenafil

During 1998 the pharmaceutical company Pfizer introduced sildenafil citrate, an oral agent for the treatment of ED known as Viagra. Sildenafil has no relaxant effects on isolated strips of human cavernosum but rather enhances the effects of NO by inhibiting the enzyme phosphodiesterase 5 (PDE5) which is responsible for the break down of cGMP in the corpus cavernosum.[5] Although there are many types of PDE in the body sildenafil is highly specific for PDE5 thus limiting any unwanted effects. Maximum plasma levels are reached in 30-120 minutes depending if the drug is taken during a meal or during a fasting state. Once metabolized, sildenafil is predominantly excreted in the feces and to a lesser extent in the urine. Sildenafil was assessed for its effectiveness on the ability of men with ED to engage in sexual activity and in many cases specifically on the ability to achieve and maintain an erection, which was assessed as sufficient for sexual activity. Sildenafil was evaluated at doses of 25 mg, 50 mg and 100 mg in 21 randomized double blind, placebo-controlled trials of up to 6 months, using a variety of study designs (fixed dose, titration, parallel, crossover etc...). Sildenafil was administered to more than 3,000 patients aged 19 to 87 who suffered from ED of various etiologies with mean duration of 5 years. Sildenafil demonstrated statistically significant improvement compared to placebo in all 21 studies. The effectiveness of sildenafil was evaluated in most studies using several assessment instruments. The primary method of assessing its function was a sexual function questionnaire (the International Index of Erectile Function – IIEF) administered during a 4-week treatment-free run-in period, at

baseline, at follow up visits, and at the end of double blind, placebo controlled, at home treatment.

At the end of the long term study 88% of the patients reported that sildenafil improved these aspects of sexual function: frequency; firmness and maintenance of erections; frequency of orgasm; frequency and level of desire; satisfaction and enjoyment of intercourse; and overall relationship satisfaction. One double blind placebo controlled study only included patients with ED attributed to complications of diabetes mellitus (n=268). The patients were started at the 50 mg dose and were allowed to change to either the 25 mg or the 100 mg. By the end of the study all patients were using either the 50 mg or 100 mg dose. On a global improvement question, 57% of sildenafil patients reported improved erections versus 10% on the placebo. A review of population subgroups demonstrated the efficacy regardless of baseline severity, etiology, race and age. Sildenafil was effective in a broad range of ED patients, including those with a history of coronary artery disease, hypertension, peripheral vascular disease, diabetes mellitus, hypertension, radical prostatectomy and many others.[5]

As effective as sildenafil has been shown to be, there still exists those individuals who do not respond to sildenafil. In addition, there are many individuals who are unable to take sildenafil because of contraindications. The most prominent contraindication is the use of sildenafil in individuals taking nitrates. Sildenafil has been shown to potentiate the hypotensive effects of nitrates. For these reasons it remains crucial to keep pursuing other avenues of treatment and not to dismiss older forms of treatment as obsolete.[5]

4.3-Vasoactive Compounds

4.3.1-Papaverine

Papaverine is a benzylisoquinoline alkaloid isolated from the opium poppy that has the ability to relax smooth muscle, particularly vascular smooth muscle. Papaverine's effectiveness as a smooth muscle relaxant originates from its intracellular actions. Firstly, it has an inhibitory effect on cyclic mononucleotide phosphodiesterase, leading to increased levels of cAMP and cGMP.[6] Secondly, papaverine blocks voltage-dependent calcium channels, thus impairing calcium influx and down regulating myosin light chain kinase.[7] All these actions lead to relaxation of smooth muscle within the sinusoids of the

cavernous tissue and the helicine arteries. Papaverine is metabolized in the liver and the plasma half-life is 1-2 hours after intracavernous injection, with peak serum levels reached within 10-30 minutes. The toxicity of papaverine is limited to two effects, firstly a local corporal fibrosis which may be due to the acidic nature of papaverine (pH 3-4), secondly hepatocellular toxicity manifested as transient elevation of transaminase. Virag's original paper[8] described bimonthly injections of 80 mg of papaverine followed by infusion of heparin solution to maintain rigidity. This treatment was beneficial in 9 out of 14 patients. Four of these patients resumed normal sexual activity without the need for additional injections. Gilbert and Gingell[20] reported on 194 men with psychogenic impotence who were using intracavernous injections of papaverine alone. Spontaneous return of erection (needing no more ICI) was seen in 21 patients and prolonged erections were found to occur in 5 cases. Some studies have shown a 17% incidence of prolonged erection associated with ICI of papaverine. Pharmacological studies in animals have shown that the effect of an intracavernous papaverine injection is similar to a natural erection. It produces an increased arterial flow, sinusoidal relaxation and increased venous resistance.[9]

4.3.2-Phentolamine

Phentolamine is a competitive, nonspecific alpha (α) adrenoreceptor antagonist. Its predominant mechanism of action is a direct effect on smooth muscle to cause relaxation. However, the exact mechanism for this relaxation is not known.[10] Phentolamine like papaverine decreases the resistance to arterial flow but unlike papaverine, phentolamine does not increase resistance to venous outflow. The plasma half-life is 30 minutes and is metabolized in the liver prior to excretion. Peak serum levels are achieved 20-30 minutes following injections. Phentolamine has been known to cause tachycardia and orthostatic hypotension when given intravenously. Nausea, vomiting and diarrhea have also been noted. These effects are rarely, if ever, seen with intracavernosal administration.

Recently, a new oral preparation of phentolamine called Vasomax has undergone early clinical study. It is currently approved for sale only in Mexico and has not achieved Food and Drug Administration approval. It appears to have only marginal efficacy and a considerable side-effect profile, with hypotension and nasal congestion as the most

frequent complaints. It does not seem to be a panacea from these early reports and likely will not displace the key position played by injectable agents for those men unable to consider oral sildenafil therapy.

4.3.3-Prostaglandin E1

PGE_1 belongs to the family of eicosanoids which represent a large group of oxygenate metabolites of polyunsaturated 20 carbon fatty acids, including prostaglandins, thromboxanes, leukotrienes and others. The eicosanoid precursor is arachidonic acid, which is found in the phospholipid bilayer of cell membranes. Arachidonic acid is released from cell membranes by the enzyme phospholiapse A_2. Eicosinoids have a wide range of effects depending on the type of receptor they bind.[11] Specific PGE_1 receptors were first detected in 1973 in the cytosol of liver cells.[9] Since then PGE_1 has been used in the treatment of occlusive peripheral arterial disease. The plasma half life is <1 minute due to rapid pulmonary clearance of up to 80% during its first passage through the lungs.

PGE_1 modulates the enzyme adenyl cyclase leading to an increase in cAMP.[12] The accumulation of cAMP leads to a decrease in the concentration of free calcium, thus impairing muscle contraction. In both human and animal studies, PGE_1 has been shown to have a relaxant effect on the arterioles, thus leading to a decrease in peripheral vascular resistance. In the venous system PGE_1 leads to constriction of the vascular wall leading to a decrease in venous outflow. These vascular effects of PGE_1 are due to direct action on smooth muscle and are independent of the presence of an endothelium.

The presence of $\alpha 1$ receptor inside the corpus cavernosum and $\alpha 2$ receptors inside the penile arteries mediates the contractile activity by noradrenaline, which is released after sympathetic stimulation.[11] Italiano et al.[22] demonstrated that PGE_1 in the cavernous tissue possessed anti-adrenergic effects via the inhibition of noradrenaline output. Therefore, not only does PGE_1 exert a direct relaxant effect on cavernous smooth muscle cells via the cAMP pathway but it also inhibits the influence of the sympathetic system. Oral preparations of PGE_1 have been studied in order to ascertain its effectiveness as a treatment for ED. Fifty one patients who had ED of various etiologies were given oral PGE_1, while others were given a placebo. The results indicated no statistically significant difference between the treatment groups and individuals receiving the oral preparation of PGE_1 had complained of side effects, especially diarrhea.[11]

A study by Linet et al.[13] was designed to evaluate the safety and efficacy of alprostadil via a dose-response study. Not only were intracavernous injections of alprostadil assessed with respect to a placebo, but the drug itself was evaluated for both safety and effectiveness by altering the dose until a minimal effective dose was established or the maximal dose of 30 μg was reached. The results showed that no individual in the study responded to the placebo, these findings were statistically significant and were evaluated by both RigiScan and clinical evaluation. There was also a statistically significant dose-response relation, with both higher clinical response rates and higher RigiScan response rates with increasing dose of alprostadil. With regards to the safety of alprostadil 23% of patients injected reported penile pain. However, only 11% out of the total number of injections caused pain (148 out of 1382). Some systemic medical events that may have been related to alprostadil occurred in 6% of the group. Most of these events effected the urogenital system and were not considered serious. The results of these studies indicate the need for individualization of optimal doses of alprostadil. The side effects were similar to those reported in other studies evaluating various formulations of alprostadil. Pain was both the principal side effect and primary reason for discontinuing with alprostadil.

It has been shown that binding of PGE_1 to a specific receptor in the cavernous tissue is the initial step in mediating erection.[12] *In vivo* studies showed that dogs had no response to intracavernous injection of PGE_1, but a full erection lasting more than 60 minutes resulted from an injection of papaverine. In addition, no receptors could be detected in the cavernous tissue of the dog.

Group	Receptor Density (fmol/mg protein)	Binding affinity (nM)
Monkeys	15.4±0.5	1.8±1
Dogs	0.52	0.13
Impotent Men	1.76±2	0.2
Control Men	8.71±2	1.3±1.1

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Figure 2. PGE_1 receptor density and binding affinity as it pertains to different populations

In humans the receptor density and binding affinity correlated well with the clinical response to intracavernous injection of PGE_1. The only disadvantage of prostaglandin E1 is pain associated with the injection site. As a result, monotherapy with PGE_1 (alprostadil) is the standard of care used by 75% of all physicians.

4.3.3.1-Intraurethral Prostaglandin E_1 (MUSE)

MUSE (alprostadil) is a pre-filled, single-use, plastic applicator containing a micro-suppository of the drug alprostadil. The stem of the applicator is inserted approximately one inch into the urethra where the medication is released. MUSE is a departure from existing therapies for erectile dysfunction, based on the discovery that certain medications can be absorbed by the urethra and transported into the surrounding erectile tissue[23]. Muse therapy consists of a proprietary, non-invasive drug delivery system that delivers pharmacological agents via the urethra and is applied into the urinary opening.

Studies have been done to compare the efficacy of intraurethral PGE_1 versus that of intracavernosal injection. One such study examined sixty men with organic erectile dysfunction whom were randomized to receive either 20 mg of intracavernosal PGE_1 (group 1, 30 patients) or 1 mg MUSE (group 2, 30 patients)[24]. Response to the drugs was recorded in the outpatient clinic and all patients continued a home-treatment program for three months. After each home administration, patients recorded the grade of erection in diaries, whether or not sexual intercourse occurred and any adverse reactions to the drugs.

Comfort and ease of administration were also recorded. Ten patients in group 1 and twenty-five in group 2 completed the 3-month treatment program, thus there was a withdrawal rate of 67% and 17% for groups 1 and 2, respectively. During outpatient dosing, 27 (90%) patients in group 1 and 18 (60%) patients in group 2 achieved a good erection. Although MUSE is less effective than intracavernosal PGE1, it is more attractive and accepted well by most patients as an easy method of treatment with minimal or no discomfort.

Another study involved MUSE and patients who had undergone penile prosthesis implantation and had later presented with malfunctioning prosthesis but choose not to undergo revision surgery[25]. Intracorporal injection therapy is contraindicated in any patient with a penile prosthesis and use of a vacuum erection device may result in prosthesis cylinder rupture. In these patients intraurethral application of alprostadil may restore prosthesis function and permit satisfactory intercourse. The study was designed to evaluate the efficacy of a medicated urethral system for erection (MUSE) using alprostadil to restore function for men with a failed prosthesis. Twenty-eight men, ages 47 to 81 (mean age 61.2) with a penile prosthesis were treated with alprostadil. Of the patients 11 had penile prosthesis failure (group 1) and 17 reported decreased glans penis engorgement (group 2). Erections were sufficient for intercourse in 7 of 11 group 1 patients, and 10 of 17 group 2 were satisfied with treatment. A significant or excellent response was noted in 10 of 18 men observed at the clinic. The researchers concluded that intraurethral alprostadil may be used to restore or improve function of a penile prosthesis in patients with a malfunctioning device or lack of glans penis engorgement.

Although there does exist clinical applications for MUSE it's usually amongst certain populations of patients who suffer from erectile dysfunction and not the general population of ED suffers. In addition the results are not as high as those of intracavernosal injections of alprostadil.

4.3.4-Endothelin-1

Endothelin-1 is a 21-amino acid peptide and is one of the most potent vasoconstrictors.[14] Endothelin-1 is also considered to be a physiological antagonist of endothelium-derived relaxing factor. Similar to other vasoconstrictors, binding and membrane receptor activation leads to an increase in intracellular calcium

concentrations.[14] Furthermore, endothelin-1 has been reported to have direct potentiating action on the contractile response of vascular smooth muscle.[14] Therefore, it is conceivable that endothelin-1 may be an important modulator of erectile physiology and dysfunction.

A balance between vasoconstricting and vasorelaxing hormones is essential for the maintenance of normal erectile function. *In vitro* studies have shown that corporal smooth muscle relaxation, induced by endogenous vasorelaxants, may be impaired in a large proportion of impotent men.[15] These *in vitro* findings are consistent with the hypothesis that the etiology of erectile dysfunction in many patients maybe the result of heightened corporal smooth muscle contractility or impaired corporal smooth muscle relaxation.

Cultured endothelial cells from human corpus cavernosum are capable of expressing endothelin mRNA, in addition the cells possess specific binding sites for endothelin-1. This suggests that endothelin-1 may play a vital role in the regulation of tone in the human penis.[14] Endothelin-1 released from corporal endothelial cells or smooth muscle might act as paracrine or autocrine factors, which can alter tissue contractility. It is possible that endothelial cell dysfunction could shift the balance between vasodilatation and vasoconstriction in the corpora cavernosa in favor of contraction. A second possibility also exists in which endothelin-1 may potentiate the release of norepinephrine from presynaptic nerve terminals within the corpus cavernosum.[15] This release would shift the balance towards higher contractility resulting in incomplete corporal smooth muscle relaxation.[15] Penile Doppler ultrasonography is being used to evaluate the flow within the cavernous arteries in patients who have received radiation treatment for prostate cancer. One of the most common side effects of radiation treatment for prostate cancer is impotency and the vast majority of patients experience this side effect. A previous study in an animal model has shown that ET-1 antagonist BQ-123 allows recovery of full erectile function (Merlin et al. personal communication). In order to ascertain the reasons and mechanisms involved, a collaborative study between Dr. G. Brock and Dr. G. Shenouda at the Jewish General Hospital was initiated to explore the hypothesis that radiation increases serum levels of ET-1, which is a very potent vasoconstrictor. Patients prior to receiving their radiation

treatment are evaluated with duplex ultrasonography. The evaluation then continues at 3 weeks, 6 weeks and 6 months following their first treatment. It is predicted that there will be a reduction in the arterial diameter as the course of treatment progresses. This reduction will translate into a lower flow velocity and hence an overall decrease in the rate of arterial inflow resulting in arteriogenic impotency.

4.3.5- Bimix Pharmacotherapy (Papaverine-Phentolamine)

Using papaverine alone as an agent for vasoactive pharmacotherapy has several problems such as delayed onset, variability in its efficacy between doses and a significant risk for priapism (persistent erection, accompanied by pain and tenderness). After extensive studies the most common dose ratio is presently 30 mg of papaverine combined with .5 mg of phentolamine.[16] Initially 250 patients received a 30mg papaverine and 1 mg phentolamine solution. Whereas only 1.6% had prolonged erections, 72% of the patients reported a satisfactory result. Due to these results, 97% of the initial 250 patients went on a self-injection program and continued to have excellent results and a low dropout rate. Papaverine, a smooth muscle relaxant and phentolamine, an alpha-adrenergic blocker has been used widely in diagnosing and treating impotency. A study was performed to evaluate the hemodynamic effects of phentolamine and papaverine using dogs.

Drug (Dosage)	Baseline Flow Range (ml./min.)	Peak Flow Range (ml./min.)	Peak Rise from Baseline Range (%)
Papaverine (30mg)	2.5-5.0	7.5-17.5	300-700
Phentolamine (5mg)	2.5-5.0	5.0-9.7	50-100

Figure 3. Injection of either papaverine or phentolamine and the effect it has on baseline flow (ml./min.)

Blood flow within the internal pudendal artery was measured after ipsilateral intracorporal injection of papaverine or phentolamine. The results showed that the percent rise from baseline was much more dramatic with the use of papaverine.

Phentolamine was only able to raise the arterial blood flow by 100% above baseline whereas papaverine at the very least raised arterial flow by 300%. To account for these changes it has been suggested that papaverine not only induces smooth muscle relaxation but arterial dilatation as well as sinusoidal wall relaxation. This results in a decrease in resistance to in-flowing blood and an increase in resistance to out-flowing blood (venous occlusion).[17]

4.3.6- Trimix Pharmacotherapy (Papaverine-Phentolamine- PGE_1)

Each vasoactive drug used in the treatment of male erectile dysfunction has limitations in its clinical success and all possess side effects. Papaverine as a monotherapy has an unpredictable response with a significant potential for priapism. Chronic injection can result in cavernosal smooth muscle hypertrophy and fibrosis. In addition there have been reports of hepatic dysfunction. The use of phentolamine as a single agent for intracavernous injection therapy does not produce an erection sufficient for sexual intercourse. Papaverine in combination with phentolamine does decrease the latency between injection and erection and allows for a reduction on the volume of papaverine that is used. Prostaglandin E1 with its short half-life and dose-dependent duration of erection has an advantage over papaverine monotherapy and papaverine-phentolamine combination therapy. However, due to the high cost of PGE_1 and its high incidence of pain that follows the injection, these factors have prompted the combination of all three drugs.

Since each agent acts on a specific site in the erection process, it is possible to take advantage of synergism at very low doses of each individual agent. Bennett et al.[18] combined 4.4-mg papaverine; .15mg phentolamine and 1.5g PGE_1 in a total volume of .25 ml. One hundred and sixteen patients diagnosed with varying causes of erectile dysfunction were given the triple drug therapy. Eighty-nine percent of the patients had a positive result and then went onto home injection therapy. Seventy-eight patients maintained the volume of .25ml with an average frequency of 3.1 injections per month. Only two cases of prolonged erection requiring treatment were seen, in both cases the individual had psychogenic impotency. Two patients complained of pain around the injection site. After 12.7 months no patient suffered from corporal fibrosis that had been seen with the other forms of vasoactive drug therapy. Nonetheless, triple drug therapy is

still reserved for those patients where PGE_1 doesn't work, or is too painful or too expensive. These sentiments were reiterated in the closing remarks at the 6th world meeting on impotence on September 1994.

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Chapter 5 - Medical Treatment

5.1-Attempts to Increase Patient Acceptance of Intracavernous Injection Therapy

Despite the literature reports of numerous complications and even death in the administering of vasoactive drugs, intracavernosal injection of vasoactive drugs has been the principal method of diagnosing and treating erectile dysfunction for over a decade.[1] In fact the use of intracavernous injection of vasoactive agents has gradually expanded its role in the treatment of erectile dysfunction. This increasing experience in the use of vasoactive agents could explain some of the complications that have emerged. Currently the vast majority of patients can be successfully treated with ICI.[1] Despite the high success rates with self-injection therapy, there still remained a high drop out rate. Patients reported that the preparation of the syringe, needle and appropriate dose had a destructive effect on their own and their partner's sexual activity. In order to solve this problem a new injection system was introduced to facilitate home injection therapy.

Until January 1991 insulin syringes were used for injections and patients were instructed in how to fill the syringe with the appropriate dose of the drug and how to perform the injection under sterile conditions.[2] The new injection device was called D-PEN and consisted of a capsule that screwed into an adapter after inserting the filled glass cartridge, finally, the needle is then screwed into the adapter.

Causes of dropout among syringe users

	Number of Patients (%)
Discontinuers:	
❖ Technical difficulties with injections	2 (3)
❖ Mixture ineffectiveness	3 (4)
Dissatisfied:	
❖ Bother of injection maneuvers	8 (12)
❖ Lack of sexual interest	7 (11)
❖ Artificiality	2 (3)
❖ Concomitant disease	2 (3)
Total	24 (36)

Figure 4. The various reasons why syringe users of ICI elect to discontinue treatment.

Causes of dropout among pen users

	Number of patients (%)
Discontinuers:	
❖ Mixture ineffectiveness	2 (2)
Dissatisfied:	
❖ Lack of sexual interest	8 (6)
❖ Artificiality	1 (1)
❖ Concomitant disease	5 (3)
Total	16 (12)

Figure 5. The various reasons why the users of the pen for ICI elect to discontinue treatment.

When shown the new device, 39 of the remaining 44 patients (90%) decided to abandon the syringe and use the new self-injecting pen.

It is evident that the self-injecting pen device had a high rate of satisfaction among the users. Most of the patients felt that the pen was more accurate in terms of dose selection and the pen was more convenient to use. Among the patients who were familiar

with both systems, the syringe and the pen, only 4 patients (4%) elected to use the syringe while the other 88 patients (96%) preferred the pen. The overall dropout rate among pen users was 12% which was significantly less ($p<0.005$) than in patients using the syringe.[2]

In spite of a high rate of satisfaction among the users of the auto-injecting devices, there still remain a significant number of men who fail to attend follow-up appointments. The Department of Genitourinary Medicine in Belfast Northern Ireland studied these patients in an attempt to identify factors that might contribute to the patient's decision to withdraw from treatment. Questionnaires were designed in order to accurately ascertain the reasons for dropping out of the program. Forty-seven patients were treated successfully, but all failed to attend their follow-up appointments. Thirty (64%) of the forty-seven patients completed the questionnaires and returned them. The results are as follows:

Reason	**Number of cases (%)**
Recovery of erectile function	8 (27)
Loss of partner	4 (13)
Deteriorating health	4 (13)
Method regarded as unacceptable	3 (10)
Reluctance to use injections	3 (10)
Failure to work	2 (7)
Difficulty with technique	2 (7)
Death of patient	2 (7)
Work commitments	1 (3)
Distortion of penis	1 (3)
Total	**30 (100%)**

Figure 6. Factors that contribute to patient's decision to withdraw from treatment.

Eleven (37%) of the thirty patients withdrew from the treatment program because of problems relating directly to the treatment and anxieties associated with the intracavernous injections. Careful analysis of the questionnaire revealed that 6 cases (20%) had problems related to injection techniques. High success rates notwithstanding,

the dropout rate for intracavernous injections of vasoactive agents remains very high. In fact, pharmacotherapy programs typically show dropout rates of 40-50%.[3]

The histopathological changes that occurred in the corpora after long-term intracavernous injections remained mostly unknown. A study was done in monkeys divided into three treatment groups, normal saline, papaverine and prostaglandin E1, to see what if any histological changes occurred after chronic injection.[4] Both light and electron microscopy was then used to evaluate the tissue.

Light microscopy of the cavernosal tissue from the normal monkey, showed loose sinusoidal spaces separated by connective tissue and bundles of smooth muscle within the trabeculae. Cavernous tissue from the saline group also showed localized aggregation of dense hyalinized collagen fibers, surrounded by hyperplasia of intracavernous components which resulted in the narrowing of the sinusoidal spaces. Monkeys treated with PGE_1 showed similar findings to the saline group. Thus, evidence of sinusoidal narrowing occupied by hyperplasia of the cavernous tissue, including fibrous tissue and increased smooth muscle mass, was found. Areas of local fibrosis were visible at the site of injection. The papaverine groups showed similar findings to the saline and PGE_1 groups, however, a variety of more severe histopathological changes were seen. These changes included hypertrophy and increased smooth muscle mass, increased collagen and scar formation around the site of injection.

Under electron microscopy the saline and PGE_1 groups were quite similar, that is normal in most aspects. More specifically there were no obvious changes in the endothelial cells, however, degeneration of smooth muscle cells and increased collagen fibers were seen next to fibrotic areas which were also seen under light microscopy. The most significant finding in the PGE_1 group, but not in the saline group, was hypertrophy of smooth muscle cells. In the papaverine group, changes in the ultrastructure were visible everywhere including different stages of degeneration of smooth muscle cells.[5] The results that were seen with the papaverine group, but not seen in the saline or PGE_1 groups, are possibly due to the acidic nature of papaverine (pH 3.2). Regardless of the agent used, localized fibrosis was observed which might emphasize the fact that intracavernous injections are traumatic.

For over a decade, intracavernous injections have been used as a highly effective diagnostic tool and therapy. One of the major disadvantages of intracavernous injection therapy is the risk of penile scarring. An experiment was conducted to evaluate possible risk factors for penile scarring that resulted from the use of PGE_1. Ninety-two patients were recruited with a mean age of 59 years with a mean follow up period of 19.5 months. These patients received a mean number of 85 injections. Penile scarring developed in 15 patients during the follow up period. These 15 patients were compared to the remaining 77 patients but no significant differences were found in terms of age, frequency of injection or follow up period.[6]

Scarring is a well-documented complication of papaverine and phentolamine injection therapy, which appears to be related to the duration of the therapy and frequency of the injection.[6] The use of PGE_1 may also result in scarring, however, when patients with scarring were compared to those without scaring no significant differences were noted in terms of duration, dose and frequency. This is in contrast with what was found with papaverine and phentolamine injections.[6]

A less invasive alternative to ICI may be the use of a no-needle jet injector. Such a device has already been used for the administration of local anesthetics, insulin injections and immunization. Seyam et al. [7] evaluated the jet injector using rats to determine the depth of penetration, impairment to the hemodynamic physiology of the corpus cavernosum and effective delivery of papaverine. These objectives were studied by using Indian ink to stain the cavernosal tissue whereas physiological function of the corpus cavernosum and the effective delivery of papaverine were evaluated by EFS studies.

Ink was found to various degrees at the level of the tunica albuginea and within the corpus cavernosum averaging 41%-49% of the cross-sectional area of the corpora cavernosa. There were no structural abnormalities seen within the cavernous tissue. The second group showed no significant pathological changes, although subcutaneous hemorrhaging was seen at the injection site in 3 of the 5 animals. The groups receiving 108 and 325 μg of papaverine showed no significant changes in ICP compared to saline. However, at a higher dose of papaverine (3250 μg) a significant rise in ICP was measured. This higher dose was needed because only a fraction of the drug reached the cavernous space. All rats in this sample population showed evidence of subcutaneous

hemorrhaging. Perhaps the no-needle jet injector maybe an alternative to needle injection, however, further studies are required and consideration must be given to achieving the appropriate dose and avoidance of subcutaneous injury.

5.2-Subcutaneous Drug Delivery Device

Not long after its inception, intracavernous injection therapy was being used in a wide variety of patients suffering from erectile dysfunction. Many studies have shown ICI therapy to be very effective and relatively safe. As time progressed and ICI therapy increased in popularity, this translated into more patients using ICI therapy as well as an increase in the frequency of injections. This gave rise to increasingly more reports on cavernosal fibrosis. Recent studies showed that cavernous fibrosis is not only caused by the injected drug itself but also by the repeated trauma sustained to the cavernous tissue by repeated punctures.[8] As a result of this side effect the idea of a subcutaneously implanted drug delivery system was born. This would allow the patient to inject the vasoactive drug into a reservoir, which could be positioned near the lower abdominal cavity or pelvic region. The patient would inject the drug into the reservoir and not into the cavernous tissue, thus eliminating any possibility of developing fibrosis.

Such an experiment was carried out in monkeys by Stief et al.[8] at the University of Freiburg. When comparing the first injection to the thirtieth injection of papaverine-phentolamine mixture, the dose needed to be increased by 240% in order to obtain the equivalent response. After 30 injections the dose remained constant for the remaining 70 injections. After 30 injections 2 monkeys were sacrificed and subjected to histological examination. A thin fibrotic layer around the implantation site of the catheter was observed. There was a non-significant increase in collagen fibers in the proximal and medial aspect of the corporal bodies. In the distal end where the valve tip catheter was placed the majority of the cavernous smooth muscle was replaced with fibrous tissue. The monkey injected 100 times as well as the monkey that was injected 88 times showed similar histological findings to the two monkeys that received 30 injections.

Stief et al.[8] concluded that a drug delivery system was a good alternative to auto-injections of vasoactive drugs, however, long-term use still needed to be examined carefully.

5.3-Special Population (Spinal Cord Injuries)

Erectile dysfunction whose origin is due to neurogenic factors such as spinal cord damage constitutes a significant proportion of men who seek treatment for erectile dysfunction. Sexual rehabilitation in spinal cord injury patients has assumed an increasingly dominant role.[9] The majority of SCI patients seeking help for their ED are young and healthy, thus lending to few if any compounding pathophysiological factors which can exacerbate their ED. Due to this fact, SCI patients constitute a valuable study group. Due to their injury, their physiology remains intact except for their neurogenic pathology; this can offer new insights into the evaluation and treatment of ED.

Many studies have been done to determine the effects of drug therapy on spinal cord injured patients. In fact the use of vasoactive agents in SCI patients for the treatment of erectile dysfunction is preferred at many medical centers due to its effectiveness and low dosage.[10] In the study by Tang et. al.[10] they reported that 93.3% of their patients had a successful erection with an average duration of 59.1 minutes, the patients received PGE_1 ranging from 0.5-20mg. These results are consistent with those reported by Waldhauser[11] and Ishii.[12] According to studies, successful sexual intercourse among spinal cord injured patients is noted at 20-30%.[13] In another study by Kapoor et. al.[9] they evaluated the role of intracavernous injection of papaverine in the management of impotency. They found that 78 out of 101 patients were able to obtain an erection 10 minutes after injection. A total of 98 patients had achieved an erection that was adequate for sexual intercourse.

Patients who suffer from erectile dysfunction as a result of a spinal cord injury have been able to choose from a variety of therapies such as penile prosthesis, vacuum devices, oral agents such as yohimbine (alpha-adrenergic antagonist) and topical nitroglycerin agents. Although rare, there have been problems associated with vacuum devices and prosthesis. As a result, a non-invasive course of treatment is usually preferred.[14] Using a standard rat model of spinal cord injury with impact trauma at the level of T10, the study done by Rivas et. al.[15] showed that the highest intracavernous pressure rise was in response to intracavernous injection of papaverine.

Centers that specialize in sexual dysfunction have a wide variety of resources available to them. These medical interventions range from vacuum devices to surgery.

Despite a few multidisciplinary approaches to sexual dysfunction in the spinal cord injured patient, rehabilitation centers have tended to focus on intracavernous injections as the sole mean of intervention.[16] Although, using a multidisciplinary approach may be the more prudent method, that is not the concern of this research. The main concern is in the effectiveness of ICI in spinal cord injured patients. In regards to this issue there is little doubt that ICI of papaverine is effective. ICI of papaverine has been found to be very powerful in overcoming erectile dysfunction.[16] According to the results of Courtois et al.[16] papaverine injections demonstrated that the drug is equally effective for subjects suffering from various lesions. A study done by V.K. Kapoor[9] showed that 78% of their spinal cord injured patients developed a good erection within 10 minutes after receiving a papaverine injection.

Papaverine is an opium alkaloid that acts directly to relax smooth muscle cells and dilate arteries, thus making it an ideal agent ideal for those patients with neurogenic erectile dysfunction.[17] There have only been a few studies that demonstrate the long-term effects of chronic intracavernous papaverine injections. The first study was done by Aboseif et al,[18] and it involved injecting monkeys with papaverine twice a week until a total of 75 injections were reached. It was noted that initially (21 injections or less) all three monkeys that were receiving 10 mg of papaverine achieved an erection that lasted 30-40 minutes. Two of the three monkeys over the next few injections had a decrease in the duration of the erection from the original 30-40 minutes to 20-25 minutes. Eventually the duration of the erection decreased to 10 minutes in both of these monkeys. The third monkey developed cavernositis after the 22nd injection, which resolved after antibiotic treatment. The animal continued to respond to the injection but with decreased strength and duration until the 52nd injection, when papaverine injection was completely unable to induce an erection.

All three monkeys were examined histologically using both light and electron microscopy. All samples showed a loss of the normal architecture at the site of injection and areas distal and proximal to the injection site. Some of the pathological changes that were seen were fibrosis with an increase in the amount of collagenous tissue, edema, degeneration and atrophy of the smooth muscle. Electron microscopy confirmed these findings and showed a complete distortion of normal histological structures. The

papaverine group did demonstrate a loss of erectile capability over time, evidenced by a marked decrease in the duration of erection in 2 out of the 3 monkeys and a complete loss of erection in one monkey. This gradual decrease in erectile response is believed to be a result of the histological changes in the corporal bodies. The most significant histological finding was the degeneration of smooth muscle and its subsequent replacement with collagen fibers. This would no doubt compromise the compliance of the sinusoids.

A study was done by Abozeid et al.,[19] in which a total of 7 animals each received 100 IC injections. These injections were given every 2-3 days over a one-year period. All monkeys showed a strong response to the treatment, however, over time their responses changed. There was an inverse correlation between the response to papaverine and the amount of fibrosis in the corpora cavernosa. This study demonstrated two important issues; firstly, papaverine is a potent erection-inducing drug that works well over the long term; and secondly, the chronic use of papaverine does induce histological changes that can lead to some loss of erectile activity over time. The pathological change that results (fibrosis) can be due to: (1) local irritation from the papaverine itself. (2) fibrosis due to the acidic nature of papaverine (pH 3.2) and (3) the inflammation response that is generated by the trauma of the injection. Although this was study was done using non-spinalized monkeys the pathological changes in the erectile tissue should not differ in a spinalized population of animals.

Finally, a study done by Rivas[15] compared different pharmacotherapies in treating animals with spinal cord injuries. The results showed that intracavernous injection of papaverine generated the greatest ICP rise. They also observed that with regard to the effect of spinal cord injury on the erectile response, SCI animals were noted to have an exaggerated erectile response when compared to control animals. It was postulated that this was due to increased sensitivity, caused by denervation as a result of the spinal cord injury itself. It is unclear whether this denervation supersensitivity develops from an increase in activity of neuroreceptors within the corporal bodies or whether the number of receptors may ultimately be increased as a result of denervation.

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Chapter 6 - Dog Experiment

6.1-Objectives

The goal of this study was to assess the reliability of a subcutaneous drug delivery system as an alternative to ICI as well as to provide direct histochemical evidence of the effects of chronic vasoactive agents on nitric oxide synthase. The objective was to demonstrate that long-term injections of a vasoactive agent such as papaverine can increase the expression of NOS as a result of the increase in penile blood flow.

6.1.1-Canine Model

The anatomy and physiology of the dog is remarkably similar to the human, more specifically the histology of the penis and the mechanism of erection is quite similar, thus allowing for inferences to be made between the two species. The dog has been used by many researchers in the filed of urology to assess different components of the erectile mechanism. As a result of a vast array of erectile experiments having been performed on dogs it was obvious that the dog model would provide accurate and useful data.

6.2-Materials and Methods

6.2.1-Chemosite™ Implantation

Our sample population was comprised of three adult male mongrel dogs weighing between 25-45 kg. Chemosite™, manufactured by Auto Suture (Norwalk, Connecticut), was the implantable drug delivery system selected for this experiment. This device was selected because of its construction and design. A pediatric model was obtained which weighed only 7 grams and had a height of 13.2 mm. The port/reservoir capacity 0.5 ml and made of titanium, the septum was made of silicone rubber and guaranteed for 2000 injections. The catheter was constructed from polyurethane and was secured to the port via a polypropylene locking mechanism. These features allowed for a flexible catheter that was strong enough to withstand the daily activities of an active adult dog.

6.2.2-Surgery

Unlike humans, dogs have a penile bone. This Os Penis limited direct access to the vascular aspect of the penis (the corpora cavernosa), making needle delivery of the vasoactive drugs difficult. Another anatomical difference lies in the dog's continuous septum, unlike the human fenestrated septum. Therefore, the dog has a right and left penis that is independent from one another. Since the catheter was inserted into the right side, the drug remained in the right side, thus allowing the left side to serve as a control.

The dogs were pre-anesthetized with 0.02 mg/kg atropine sub-cutaneously, 0.2 mg/kg acepromazine intramuscular and 0.01 mg/kg buprenorphine subcutaneously. After this initial state, 10-15 mg/kg somnotol was given intravenously as needed. An endotracheal tube was placed to allow for free ventilation.

The animal was placed in a supine position and a mid-line lower pelvic perineal incision was made. Via blunt and sharp dissection the base of the penis was exposed. The urethra was identified and an incision was made through the tunica albuginea into the corpus cavernosum. The catheter was then inserted 2-3 cm into the cavernous tissue in the base of the penis. The tissue was then over sewn with 4-0 silk sutures. The pelvic incision was then extended and the iliac arteries were exposed. In man the abdominal aorta branches into the common iliac arteries. Each common iliac artery then bifurcates into the internal and external iliac artery. In the dog the anatomy is somewhat different with respect to the bifurcation. The dog's common iliac trifurcates therefore, it was decided that in order to create arterial insufficiency in the penis, the common iliac artery was partially occluded bilaterally. Occlusion was achieved by tying a plastic rod to the common iliac. Once tied, the rod was removed leaving a ligature around the artery with a diameter equal to that of the rod (approximately 1.3 mm). The pelvic wound was then closed in two steps. The abdominal muscles were sutured using 2-0 catgut in a non-interrupted fashion. The outer more superficial layer of skin was closed using 4-0 absorbable suture material such as biosin or polysorb in an interrupted fashion.

Next a 2-3 cm incision was made between the scapula of the dog to allow for insertion of the reservoir. Once in place the reservoir was anchored by three 4-0 silk sutures. The catheter was manipulated from the base of the penis to meet the reservoir. This was done by subcutaneously tunneling the catheter from the base of the penis to the

scapula. Post-operative treatment consisted of antibiotics for 5 days and careful observation of the animal. The dogs were allowed three weeks to heal before any injections were given.

Chapter 7 - Chronic Intracavernous Injections in the Spinalized Rat

7.1-Objectives

The rat has been used in many studies in order to assess penile erectile function. The rat model is an ideal animal for the study of erection for many factors, and due to its similar anatomy direct intracavernous injections could be accomplished. Due to concerns that repeated intracavernous injections would be painful for the experimental animals, it was decided that spinal section surgery should be undertaken in order to cause somatic sensory denervation.

7.2-Materials and Methods

The simplified anatomy of the rat allowed for a wide variety of experimental procedures to be carried out. The major pelvic ganglion is located bilaterally on the dorsolateral lobes of the prostate. It receives its innervation from L6 and S1 via the pelvic nerve and from the thoracolumbar outflow via the hypogastric nerve. The cavernous nerve exits the major pelvic ganglion and runs along the lateral aspect of the urethra without branching. Tumescence is easily produced upon stimulation of the cavernous nerve. Electric field stimulation of the cavernous nerve is limited to the corpora cavernosa (shaft and bulb).

Mating studies are easily performed in the rat allowing for a natural way to assess and evaluate erectile function and performance. Due to low cost, housing and availability, studies involving a large sample population can be done with relative ease. The number of people required to perform surgical procedures on a rat is significantly less than what is required for a larger animal such as a dog. In addition the time it takes to perform a surgical procedure in a rat is usually significantly less than that required for large animals. These factors allow one or two individuals to carry out a complete experiment without the assistance of additional personnel.

7.2.1-Rat Surgery

The animals were spinalized at the level of the tenth thoracic (T10) vertebrae under microscopic vision. This procedure was done under anesthesia using a combination of 50 mg/kg ketamine and 5 mg/kg xylazine. The animals were pre-medicated with antibiotics (tribrussin 0.01mg/kg) and fluid was given subcutaneously to assure adequate hydration. Once a deep state of anesthesia was achieved, a 5 cm vertical incision was made on the dorsal surface overlying the spinal cord. Once the incision was made and T10 was identified using the last rib as a landmark, the inter-vertebral space was exposed. Under a dissecting microscope the spinal cord was visualized and a small section of bone from the vertebra was removed, thus creating a window. With the spinal cord now visible within the vertebral canal the transection could be accomplished. Using a suction device, a small segment of the spinal cord was severed. Still using the microscope the two ends of the spinal cord were viewed in order to ensure a complete and uninterrupted transection of the cord prior to closing the wound.

The muscle layer was sutured using 4/0 polysorb and the skin was sutured using 4/0 dexalon. The procedure was carried out under complete aseptic conditions and the animals were monitored until they regained consciousness. Pain management was effectively achieved post operatively by administering buprenorphine (.01mg) twice daily for 2 days post-operatively. As a result of the trauma to the spinal cord the rats were unable to evacuate their bladders for 3-4 weeks post-operatively. This meant that all the rats had to have their bladders manually emptied three times a day for one month post-operatively. Manual bladder expression was accomplished by locating the dome of the bladder between the thumb and middle finger. Once this was done gentle but constant pressure was applied until the bladder was completely emptied of its contents.

Thirty animals had undergone the surgery, however, only 50% of them survived the total duration of the study. Eleven of the animals died within the first two weeks, following the surgery. The major reason for their deaths was severe hematuria, which was seen in all animals immediately following the surgery. Autopsies of the dead animals revealed many abnormalities; the most common and pronounced pathological finding was severely enlarged bladders. This condition was due to hematuria, which leads to massive amounts of blood clots within the bladder. Bladder stones were also seen in some

animals, in fact one animal had well in excess of 24 stones. Large pockets of pus and fluid within the abdominal cavity, hyperplasia of the prostate and abnormalities in the gastrointestinal tract were also common in these spinalized animals.

7.2.2-Intracavernous Injection Procedure

The study objectives were to simulate conditions similar to those in the human clinical setting. The animals (papaverine and saline groups) were injected approximately 2.5 times a week. Using a 30G needle a diluted solution of papaverine in a total volume of 0.1ml was injected into the cavernous tissue of the rat, in the penile midshaft region. The papaverine was taken from a stock solution of 65-mg/2 ml and was diluted with normal saline to a concentartion of .001 mg/ml and injected in a .1ml preparation. The saline group as received injections of .1ml of saline in the same manner as the papaverine group of animals. Prior to injecting, the rat was placed in a plastic restrainer and immobilized. The penis was exposed and disinfected with iodine. The needle was inserted on the lateral aspect of the shaft avoiding the vein and urethra. Once the injection was completed, pressure was applied in order to control any bleeding and prevent the formation of hematoma. The penis was disinfected once again and the rat was returned to his cage. Prior to any injection the physical condition of the rat was evaluated including the condition of the penis. If there were any visible signs of distress or if the penis had a hematoma, injections were postponed until the next day providing the situation had improved.

Our sample population had received 10 to 15 injections in an 8-week period or less. The only complication that was observed as a direct result of the injections was the occurrence of hematomas in two of the animals. These hematomas resolved themselves and the injections were continued.

Chapter 8 - Results

8.1-Dog Experiment

8.1.1 Measuring Intracavernous Pressure

Using a 23G-butterfly needle attached to a pressure transducer, which was connected to a Grass Stimulator, the intracavernous pressure was assessed. The needle was inserted into the port, which provided the ability to assess the cavernous pressure. Since the catheter contained no valve, the pressure within the cavernous tissue was equal to the pressure within the catheter and port. Initial readings were taken to ascertain base line levels. According to Juenemann et. al.[1] they determined the basal intracavernous pressure of the dog to range between 2-10 cm H_2O.

While trying to assess baseline values for the dogs, it was evident that the pressure was significantly higher than that which was stated in the literature. All three dogs had an abnormally high intracavernous pressure reading. It was initially suspected that a possible clot had formed and was obstructing the lumen of the catheter or that a fibrotic layer had formed around the tip of the catheter. The drug delivery system was flushed with 3 ml of heparinized saline (250 IU/ml). The fluid entered with little resistance and after several hours the pressure was reassessed. The pressure measured on different days varied from normal baseline values to 5 times normal.

Upon injection of small amounts of saline (0.5-1ml), there was a significant and sustained rise in pressure. Injections of papaverine ranging from 5-30 mg had no effect. It was evident from these results that there was a substantial problem with the drug delivery system in view of the fact that 3 ml of saline caused a large and sustained pressure rise whereas 15 mg of papaverine had little to no effect. In fact, according to the literature 15 mg papaverine caused a pressure rise between 110-220 cm H_2O.[1] It was decided that the dogs would undergo exploratory surgery to determine the reason for these abnormal results. Based on the evidence that had been obtained thus far it was suspected that the catheter had slipped out of the cavernous tissue and was resting between the tunica albuginea and the subcutaneous tissue.

8.1.2-Exploratory Surgery Findings

The dogs were prepared for surgery in the same manner as previously described. An incision was made slightly superior to the base of the penis and on the contralateral slide to where the catheter was initially inserted. The catheter was found and its path was traced inferiorly to the base of the penis. The distal tip of the catheter was no longer in the cavernosal tissue but it had migrated out of the corpus cavernosum and was lying superior to the tunica albuginea. This confirmed the suspicion and showed that the drug never reached the cavernous tissue and that the saline accumulated in the space between the tunica and the subcutaneous tissue. Therefore, when saline was injected the fluid remained within a confined area resulting in pressure on the distal end of the catheter and hence a significant rise was indicated on the Grass Stimulator.

In order to avoid this problem the technique was modified for inserting and maintaining the catheter in its proper place. The distal end of the catheter was anchored into the cavernous tissue by inserting a 4-0 silk suture through the tip of the catheter. This suture was then tied into the cavernous tissue holding the catheter in place. This technique was performed on two of the three dogs. The animals were allowed to recover for one week before being re-evaluated.

Upon assessing the ICP and injections of saline it was clear that the modified technique had not been successful. High base line intracavernous pressure readings were obtained as well as high and sustained pressure increases were obtained with 2ml heparinized saline injections. After several months of trying various methods to obtain a suitable model, it was apparent that the dog was not appropriate for a chronic study such as this. Prior to being sacrificed the drug delivery system was removed and the penis was examined. We noticed that in both dogs who had undergone the modified surgery that the catheter had once again slipped out of the cavernous tissue and was lying subcutaneously on top of the tunica albuginea.

Still wanting to determine the effects of chronic injections of vasoactive agents on nitric oxide synthase another animal model had to be developed. After reassessing the previous model and carefully examining what went wrong, it was evident that because of technical problems the experiment was unable to produce any substantial results. The only conclusion was that a subcutaneous penile drug delivery system was unable to

function properly in a dog. However, these results may not correlate with other animal models or humans. As a results another animal model was constructed and the second part of this study used male Sprague-Dawley rats. The rat has a physiology and anatomy that is more similar to humans in that the drug can be directly injected into the penis similar to how it is done in men. The pain that the animal could experience at repeated penile injection was avoided by using paraplegic rats. Hence, we would simulate spinal cord injury in human cases.

8.2-Rat Experiment

8.2.1-Electric Field Stimulation Procedure

Twelve rats underwent electric field stimulation to evaluate their physiological response to electrical stimulation of the cavernous nerve. This study consisted of monitoring the rise in intracavernous pressure (ICP) which was induced by cavernous nerve EFS and was performed as follows: A 2 cm mid-line lower abdominal incision was fashioned and the cavernous nerve exposed via sharp and blunt dissection. Once exposed, the nerve was hooked onto a platinum bipolar electrode. The electrode was connected to a custom made computer platform based on Labview program (National Instruments Co., Austin Tx.), which generated the stimulation current. The internal carotid artery in the neck was exposed via micro-surgical techniques and cannulated with PE-50 tubing which was connected to a pressure transducer (42587-05 Abbott laboratories, North Chicago, Illinois). A 1 cm incision was made in the perineum and the crura of the penis was exposed. A 23 gauge needle was placed in the right crus connected via PE-50 tubing to a second pressure transducer. These transducers were connected for simultaneous systemic blood pressure (BP) and intracavernous pressure monitoring. Three to five consecutive EFS of the cavernous nerve were carried out and the resulting cavernous pressure rises were recorded.

8.2.2-NADPH Diaphorase Staining

Tissue samples were harvested from the animals 15 minutes after they underwent their last EFS. The penis was cut at the most proximal end in order to ensure that the crura were harvested as well. Once cut, the glans penis was removed distally and the crus

was removed proximally. The shaft was then cut to the appropriate length. Any muscle tissue that was attached to the crus was removed and both the shaft and crus were placed into a cold freshly prepared solution of 2% formaldehyde, 0.002% picric acid in a 0.1 M phosphate buffer, pH 8.0. The tissue remained in solution for four hours. The tissue samples were then cryo-protected for 24 hours in a cold 15% sucrose solution in 0.1M phosphate buffer, pH 8.0. After the 24-hour period the samples were embedded in O.C.T. embedding compound and frozen in liquid nitrogen and stored at –70°C. The samples were then sent to GenPath Laboratories for cutting and staining for NADPH.

NADPH diaphorase was used on histological sections. The sections were evaluated in a standard fashion as indicated in most published reports[2]. Sections were obtained from both the penile shaft and the penile crus. Initially the exact identity of the enzyme producing NADPH diaphorase was not known, however, the use of biochemical studies have shown that the enzyme responsible for this histochemical reaction in neurons is NOS.[5] In confirming these findings it has been shown that all NADPH diaphorase positive neurons exhibit NOS immunoreactivity and contain NOS.[6] NADPH diaphorase positive neurons signal the presence of NOS-positive nerve fibers which are easily seen as a highly localized dense blue region. The staining pattern was assessed by counting the number of NADPH-positive nerve fibers present in 5 random fields (magnification 400X) in both the penile shaft and the penile crus. Two observers, both of whom were blinded to the origin of the specimen, counted the fields. The average value obtained between the two observers was the reported value.

8.2.2-EFS Stimulation and NADPH Diaphorase Staining

RAT #	ELECTRIC FIELD STIMULATION	NADPH STAINING	GROUP
Rat #2	✓	✗	Papaverine
Rat #8	✓	✓	Saline
Rat #11	✓	✓	Papaverine
Rat #14	✓	✓	Saline
Rat #20	✓	✗	Saline
Rat #21	✓	✓	Control
Rat #23	✓	✗	Control
Rat #24	✓	✓	Papaverine
Rat #26	✓	✓	Papaverine
Rat #30	✓	✓	Saline
Rat #31	✓	✓	Control
Rat #32	✓	✓	Saline
Rat #33	✓	✓	Papaverine

✓ = Procedure was performed

✗ = Procedure was not performed

Figure 7. EFS was carried out on all 13 animals and tissue was harvested for NADPH diaphorase staining from 10 of the 13 animals.

8.2.3-EFS Results

EFS was done on the entire sample population of 13 male rats. Each rat was stimulated 3-5 times with the exception of rat #31 who only underwent 1 EFS. The change in ICP was determined for each EFS for all 13 rats and a Student's T-test was performed to see if the results were significant. There was no statistically significant difference between any of the three sample populations.

Rat #	Group	Mean Δ in ICP (cm/H_2O)
8	Saline†	35
14	Saline	47.3
32	Saline	45.5
20	Saline	10
21	Control††	45.3
23	Control	40.7
31	Control	6
2	Papaverine†††	47.2
11	Papaverine	22
24	Papaverine	16
26	Papaverine	56.7
33	Papaverine	39.4

†Standard Deviation (SD) 17.2 and Standard Error of the Mean (SEM) 8.6

††SD 21.5 and SEM 12.4

†††SD 17 and SEM 7.6

Figure 8. The average change in ICP as assessed by EFS for the three different groups. The change in ICP represents the difference between the basal ICP immediately prior to stimulation and the maximal ICP that was reached during the stimulation. Standard deviation and standard error of the mean are indicated and calculated per group.

8.2.4-Histological Results

The histological sections were examined using light microscopy in order to view the positively NOS stained fibers by NADPH. The tissue was not evaluated to determine the effects of chronic needle injections on the architecture of the cavernosal tissue. The aim of the study was to determine if injections of a vasoactive substance such as papaverine could increase the number of NOS fibers within the penile tissue.

8.2.4.1-Penile Shaft

The results for each of the three groups (papaverine, saline and control) were pooled and a Student's T-test was performed to determine whether any of the groups were

statistically different. Comparison of the control and saline group indicated no significant difference. However, a significant difference was obtained when the papaverine group was compared to the control and to the saline. Hence, the animals receiving papaverine had a statistically significant higher number of positively NADPH stained fibers within the penile shaft.

Penile Shaft:

Group	Rat #	Field #1	Field #2	Field #3	Field #4	Filed #5	Average
Saline†	Rat #8	4	6	4	2	4	**4**
Saline	Rat #14	3	3	6	4	2	**3.6**
Saline	Rat #30	4	4	3	3	5	**3.8**
Saline	Rat #32	5	5	7	6	4	**5.4**
Control††	Rat #21	3	5	3	2	2	**3**
Control	Rat #31	5	4	4	3	3	**3.8**
Papaverine†††	Rat #11	10	7	14	9	11	**10.2**
Papaverine	Rat #24	6	5	10	8	7	**7.2**
Papaverine	Rat #26	4	5	7	4	8	**5.6**
Papaverine	Rat #33	6	3	3	2	4	**3.6**

†SD 1.4 and SEM .1

††SD 1.1 and SEM .4

†††SD 3.1 and SEM .7

Figure 9. Five cross sectional fields of the penile shaft were examined under the light microscope (400x) and the average number of NOS positive fibers were used for statistical evaluation. Standard deviation and standard error of the mean are calculated per group.

8.2.4.2-Penile Crus

Due to the method in which the penile crura were sectioned, some specimens were not usable, namely Rat #8 and Rat #21 (saline and control, respectively). As a result the sample population was too small for statistical testing.

Penile Crus:

Group	Rat #	Field #1	Field #2	Field #3	Field #4	Field #5	Average
Saline†	Rat #8	-	-	-	-	-	-
Saline	Rat #14	8	8	5	3	6	**6**
Saline	Rat #30	10	6	19	4	21	**12**
Saline	Rat #32	4	5	5	1	1	**3.6**
Control††	Rat #21	-	-	-	-	-	-
Control	Rat #31	14	17	13	8	7	**11.8**
Papaverine†††	Rat #11	7	6	5	5	3	**5.2**
Papaverine	Rat #24	8	4	3	5	5	**5**
Papaverine	Rat #26	8	5	6	6	3	**5.6**
Papaverine	Rat #33	4	4	3	2	5	**3.6**

†SD 5.8 and SEM 1.5

††SD 35.3 and SEM 15.8

†††SD 1.7 and SEM .4

Figure 10. Five cross sectional fields of the penile crus were examined under the light microscope (400x) and the average number of NOS positive fibers were counted. Upon histological examination, two slides were excluded. The standard deviation and standard error of the mean are calculated per group.

8.3-Interpretation of Results

The objective was to show direct histochemcial evidence that an alteration in the hemodynamic physiology of the penis would result in an upregulation of the enzyme NOS. In turn this may lead to increased production of NO enhancing the erectile response. The experimental design set out to show that an alteration in blood flow results in shear stress being placed on the arteries, as a compensatory mechanism the amount of NO produced is increased. This is accomplished through a direct increase in the amount of NOS and an increase in number of NOS fibers measured histologically in the shaft of the penis. Specifically the cross sections of the papaverine group showed a statistically significant increase in the amount of NOS positive fibers as compared to saline and control groups. Statistical calculations on the penile crus were not done because the

sample population was not large enough to yield valid results. The increase in positively stained NOS fibers not only supports the hypothesis but also validates other researches who have shown that alterations in penile blood flow leads to an increase in NO.[3,4]

EFS studies revealed no significant difference among the groups. A possible explanation for this occurrence can be due to the fact that despite having a greater amount of positively stained NOS fibers, the animal's physiology due to the spinalization was altered. The vast majority of the animals in our study population did have abnormal pathologies ranging from accumulation of pus, ascites and prostatic hyperplasia. It is these abnormal conditions that might have prevented a strong EFS response despite increased NOS fibers. One possible mechanism could have been that the spinalized state limited the amount and availability of essential co-factors that are needed for the conversion of L-arginine into NO by the enzyme NOS.

Despite the lack of statistical significance among the groups during the EFS trials, we demonstrated that high penile blood flow leads to upregulation of NOS fiber expression. The implication of these findings impacts not only the field of urology but other areas of medicine as well. Patients suffering from peripheral vascular diseases such as hypertension and atherosclerosis may also benefit from therapies, which induce high vascular flow. Upregulating of NOS can produce vasodilatation, which would diminish the aforementioned pathologies. As for individuals with ED, therapeutic approaches can be developed to specifically target the upregulation of NOS potentially leading to an increase in NO and better erections.

Chapter 8 - References

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Chapter 9 - Discussion

9.1-Overview

ED is a major problem that affects millions of men in North America. In the United States ED accounts for more than 400,000 outpatient visits and 30,000 hospital admissions.[1] In the majority of patients, abnormal vascular responsiveness is the underlying cause of ED and failure to retain blood within the sinusoids is the most common cause of vasculogenic impotence.[2,3] As a result, failure to achieve an erection can be due to the lack of smooth muscle relaxation within the corporal tissue. Since the discovery of endothelium-derived-relaxing factor in 1980 and its subsequent identification as nitric oxide numerous researchers have claimed that NO is the most important mediator in smooth muscle relaxation.[4,5] It has been shown that corporal smooth muscle relaxation can be induced by stimulation of NANC neurons. Nitric oxide is released from the endothelium, nerve terminals and smooth muscle cells.[6,7] Once penetrating the cell, nitric oxide activates guanylate cyclase to convert guanosine triphosphate (GTP) into cyclic guanosine monophosphate (cGMP), which signals protein kinases, ion channels and possibly other effector systems to cause smooth muscle relaxation.[8]

Nitric oxide synthase exists as a constitutive enzyme that is present under normal physiological conditions and as an inducible form which maybe expressed following immunological stimulation.[9] In the penis itself, antibodies to NOS stains efferent axons from the cavernous nerves which innervates the trabecular meshwork of the corpus cavernosum. Bilateral cavernous nerve transection abolishes staining of NOS-containing penile neurons, but not that of vascular endothelium, which could also participate in the control of erection by NO.[10] In addition the vascular endothelium also participates in the control of erection by NO. Staining of NOS has also been seen in the endothelium and adventitial innervation of the dorsal penile artery and the intracorporal network of helicine arteries.[9]

The results reported here have shown that the population that received injections of papaverine had a statistically significant higher number of positively stained NOS

fibers. We believe that due to the vasoactive injections, the penile blood flow was modulated resulting in an increase in penile blood flow. This high rate of flow placed stress on the endothelium and reacting to relieve this stress a greater amount of NO was released.

It is a known fact that during an erection there is a substantial increase in blood flow within the corporal bodies.[11] The hemodynamic forces resulting from this large surge of blood produces two forces, shear stress and pressure stretch.[12] Shear stress is a frictional force that results from the blood flowing over the endothelial surface. Pressure stretch is a force that acts perpendicular to the vascular wall. Recent evidence has suggested that shear stress acting on the endothelium results in the release of NO.[13,14]

From their experiments, Kanai et al.[12] concluded that shear stress causes Ca^{2+} dependent transient release of NO in which the amount or peak rate is dependent on the level of shear stress. In addition to the shear stress, experimental data from Saenz de Tejada et al. [15] have shown that oxygen tension regulates the NO pathway. Essential to the erectile process is the relaxation of the trabecular smooth muscle, which is associated with increasing levels of cGMP that is in turn mediated by nitric oxide.[16]

The conversion of L-arginine to NO requires oxygen as a substrate and it has been reported by Furchgott and Zawadzki[4] as well as other investigators that blood vessels that responded to NO were inhibited at low oxygen tension.[15,17,18] Saenz de Tejada[15] concluded that oxygen tension does play an active role in regulating penile erection in the following manner. When the penis is in a flaccid state the oxygen tensions in the corpus cavernosum are close to the venous PO_2 levels. At this low level of oxygen, synthesis of NO is inhibited, thus preventing smooth muscle relaxation. During tumescence arterial vessels undergo dilatation, thus increasing intracavernosal blood oxygen content by the increased flow. In this high oxygen content environment, nerves and the endothelium can synthesize NO and hence achieve smooth muscle relaxation. It was also shown that oxygen has a regulatory role in the function of NOS. In rabbit corpus cavernosum, hypoxic conditions caused a significant reduction in NOS activity. Therefore, low oxygen tension suppresses enzyme activity and this suppression is also seen in the cerebellum and macrophage systems. [19,20] The data from Saenz de Tejada et al.[15] clearly suggests that reduced oxygen tension can be a rate-limiting factor for NO-mediated relaxation in penile

corpus cavernosum regardless of the normal or pathological state of the nerves and endothelium.

The arterial as well as the venous supply are essential constituents of the erectile process. For many years it was thought that the venous system played no role in the erectile process and most people paid attention to the arterial system. However, it is now known that the venous system figures prominently in penile tumescence. Any compromise to the venous system can result in impotency.

A study was carried out by Aboseif et al.[21] to elucidate the effects of venous leakage on erection. The results showed that as the needle size increased there was a drop in ICP, however, with the venous leakage there was a compensatory increase in arterial flow. When the venous leakage was large (22-16 gauge) it exceeded the compensatory mechanism of the arterial system. Coupling venous leakage with a reduction in arterial flow impaired the erectile response.

It is important to note that during penile erection rigidity can be prevented by excessive cavernous vein outflow.[2] Therefore, when the compensatory arterial flow mechanism is overwhelmed by venous leakage, the result is erectile dysfunction due to inadequate venous occlusion. The relation between the arteries and veins is of paramount importance in order to produce and sustain a rigid erection. In the flaccid state, the sinusoidal smooth muscle is contracted and thus contains very little blood. During the erect state the sinusoidal spaces become enlarged due to the tremendous amount of arterial inflow. As a result the subalbugineal venular plexus becomes compressed against the non-compliant tunica albuginea.[23]

Although the causes of ED can be numerous and may be exclusively caused by one component, the treatment has generally been focused on one particular aspect, namely to increase blood flow to the sinusoidal spaces of the penis. For this reason the amount of research on various vasoactive agents has been quite high. The initial insight into vasoactive agents as a treatment for ED came about in the early 1980's when Virag[24] and Brindley[25] reported that ICI of vasoactive agents resulted in penile erection.[26] Once the effectiveness of these vasoactive agents were established, the next step was to elucidate the pharmokinetics and to establish what concentration of these agents remain in the corpus cavernosum and peripheral circulation. Venous samples were taken from the

corpus as well as the peripheral circulation at various staggered intervals. It was shown that papaverine decreased steadily in the corpus cavernosum over time (760g to 110g in 55 minutes). In the peripheral circulation papaverine appeared in small concentrations. Prostaglandin E1 showed a marked decrease in the corpus cavernosum from 300 to 12 ng/ml during a 55-minute period. However, no peripheral PGE_1 was detected.[27]

Clinically these results indicate that papaverine is released slowly from the corpus cavernosum. Due to the lack of local metabolism, papaverine concentration can remain high for a prolonged period of time, thus increasing the risk for prolonged erections and priapism.[27] Conversely, it will appear in the peripheral circulation in small quantities over a long period of time, thus reducing the risk of side effects. Prostaglandin E1 is metabolized locally in the corpus cavernosum. The relatively low risk of priapism is most probably due to this fact. In the periphery, side effects are unlikely due to clearance during lung passage.

One group of individuals that are particularly susceptible to ED are men with spinal cord injuries. The penis is innervated by two sets of nerves: autonomic (sympathetic and parasympathetic) and somatic (motor and sensory). Sympathetic and parasympathetic nerves merge to give rise to the cavernous nerve. The cavernous nerve runs through the corpus cavernosum and spongiosum. The cavernous nerve controls the neurovascular events during erection and detumescence. The somatic nerves are primarily responsible for sensations of the penis.

The parasympathetic nerve fibers to the penis arise from neurons located in the intermediolateral cell columns of the 2nd, 3rd and 4th sacral spinal cord segments.[8] The sympathetic nerve originates from the 11th thoracic to the 2nd lumbar spinal segment. Upon stimulation of these nerves it was observed that the sympathetic trunk causes detumescence and the parasympathetic trunk causes tumescence. It is now evident that depending on where the spinal lesion is located, erectile function may be spared. In 1971 Comarr et. al.[39] reported that 95% of patients with complete upper cord lesion are capable of erection (reflexogenic), whereas only 25% of those with a complete lower cord lesion can attain an erection (psychogenic). It is clear that the degree of erectile dysfunction is related to the extent and level of the injury.

Although a multidisciplinary approach to the treatment of sexual dysfunction in spinal cord injured patients would be most advantageous for the patient, most rehabilitation centers focus treatment on intracavernous injection therapy as the sole means of intervention.[29,30] In a study involving 66 men, of whom 52 completed the protocol, intracavernous administration of papaverine as a single agent, or in combination, was effective in inducing erection in all 52 men.[31] In another study involving 16 paraplegics and 4 quadriplegics on a self-injection program of papaverine alone or with phentolamine, 19 were able to obtain an erection adequate for penetration.[32] From these studies, as well as others, it is apparent that ICI therapy is a suitable and successful treatment option for individuals suffering from ED as a result of spinal cord injury.

Despite the extensive amount of research in urology with specific regard to erectile function and impotency, there remained an area that lacked investigation. The involvement of NO and NOS in the erectile process is well documented as are the effects of vasoactive agents on erectile function. However, no study has focused specifically on the effects of chronic vasoactive (papaverine hydrochloride) injection therapy - more precisely, the effects of chronic ICI therapy directly on NOS. The effects that ICI has on the penile tissue itself, in terms of scarring and changes to the composition to the tissue of the corpus cavernosum is known. We also know the effects that ICI has on the erectile response to EFS. Researchers have also looked at the distribution of nitric oxide-containing nerve fibers in the corpus spongiosum and cavernosum.[33] Nonetheless, a chronic model of ICI therapy, in which the primary goal is to examine the level of positively stained NOS nerve fibers, has never been done. This research is also original in the use of a sample population of spinalized rats. Unlike other research involving the short-term use of paraplegic animals, our study required that the animals be kept for significant lengths of time (3-4 months). Thus, this work is original in both aspects. Therefore, as is true for all novel research, it is expected that there would be obstacles along the way. Some were anticipated, while others were unforeseen.

The goal was to provide direct evidence via histochemistry that vasoactive therapy does increase the number of fibers that contain NOS. We were successful at showing a statistically significant ($p<0.05$) difference between the control group, saline

injected group and the papaverine group. The penile shaft of the papaverine-injected animals had a greater number of positively stained NOS nerve fibers. This provides direct and conclusive evidence that ICI therapy upregulates NOS expression. In turn there is a larger amount of NO being produced, which is essential in the erectile process.

9.2-Continued Studies

Although this study has provided evidence that vasoactive therapy does upregulate NOS expression, there are still several avenues that should be further explored. Assessing the activity of NOS via a citrulline-arginine assay in the various groups would prove to be very valuable. Perhaps ICI therapy might upregulate the activity of NOS in addition to upregulating the level of NOS. Western and Northern blots would be useful to see what effect ICI therapy has on the level of NOS mRNA and to quantify the amount of protein present.

The conclusions of these studies are exciting in that they clearly demonstrated an increase in the amount of postively stained NOS fibers in the penile tissue after the animal had received a vasoactive agent (papaverine). We were limited by our methodology which utilized spinal animals, in which there may be inherent variables related to the spinal lesion. An ideal model to explore this question would be the rat model without spinalization, closely approximating the human condition in non-spinal injury patients. Therefore the results of our experiemnts may pertain only to the spinalized population and therefore, similar studies using non-spinalized animals should be done.

9.3-Future Direction

Although ED is not a life threatening condition it is a life altering condition, not only for the male but for his partner as well. In 1994 the National Institute of Health estimated that there were 30 million North American men suffering from ED and half of them are under the age of 65. This statistic is coupled with the fact that we have an aging baby boomer population, in which there are 79 million American baby boomers that have began to turn 50 at a rate of 1 every 8 seconds. There is no doubt that ED will be affecting

the life style as well as quality of life of more and more individuals'. For this reason treatment options must exist and that means more research must be done.

The wide spread attention that sildenafil and ED have been receiving illustrates the large number of men that are affected by ED and just how severe a problem ED is in our society. The treatment and forms of therapy for ED are changing dramatically. In the past topical creams, intraurethral medication, prosthesis, surgery and vacuum devices have been used with limited success. The most successful form of treatment is currently ICI of vasoactive agents. Many studies have shown that although effective, ICI has several negative features which forces people to seek other options or no treatment at all.[34,35,36] It is not surprising that the newest form of treatment for impotency is in the form of an oral pill. Any other form of treatment cannot surpass the convenience of a medication that is taken orally. Also Sildenafil's mode of action is somewhat different than the vasoactive drugs like papaverine, phentolamine and PGE_1. Sildenafil exerts its affects by targeting cGMP. More specifically it prevents its breakdown by inhibiting the actions of phosphodiesterase type 5 (PDE5). According to an article published in Time magazine,[38] Sildenafil works by suppressing the effects of PDE5, a naturally occurring enzyme, which causes an erection to subside after orgasm by breaking down cGMP. We know that cGMP plays a vital role in the erectile process and its effect in achieving an erection is well documented.[37] By preventing the breakdown of cGMP the smooth muscle within the cavernous tissue can remain in a relaxed state, thus allowing for the sinusoidal spaces to remain full of blood. It is important to note that the erectile process involves a cascade of events that must transpire and a defect in any of the steps will result in erectile dysfunction. Nitric oxide is still a crucial element in this cascade, for it is NO that will penetrate the smooth muscle cells and activates guanylate cyclase, which in turn converts GTP into cGMP. Upregulation of NO will result in the production of more cGMP and subsequent smooth muscle relaxation.

Chapter 9 - References

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Chapter 10 - Conclusion

The goal of this research was two-fold in nature; the prime objective was to document and provide direct histochemical evidence of upregulation of NOS in a group of animals injected with papaverine over a period of time. The second objective was to evaluate the use of an alternative drug delivery method for the treatment of impotent patients. Intracavernous vasoactive drug injection has an important role in the management of patients with ED. Fear of direct needle injection into the corporal body is an important cause contributing to the drop out rate from ICI programs. For the impotent patient, alternative delivery methods is essential in order to have a higher success rate in the treatment and management of ED.

Working with dogs was an excellent way to test the efficacy of a subcutaneous drug delivery system while simultaneously evaluating the biochemical effects that the treatment had on NOS. Unfortunately, the experiment had to be terminated due to problems arising from the inability to accurately assess if the drug was being properly delivered into the cavernosal tissue.

Working with rats was ideal due to the nature of our experiment. Having a paraplegic animal required constant care because the animal could no longer take care of itself. Voiding the bladder and bathing the animal were only some of the requirements in order to ensure the health and welfare of the animal. Despite providing the animal with extensive care, the long-term survival rates from this type of surgery were approximately 50%. Nonetheless, this provided us with an adequate sample population for our study. We concluded that there was a statistically significant increase in the amount of positively stained NOS fibers in the cavernosal penile tissue of the rat.

Therefore, it can be concluded that administration of vasoactive agents such as papaverine do not only function to dilate the arteries within the penis but also increase the amount of NOS within the region. This translates into more NO being produced and hence the cascade of events that take place during erection is augmented. This experiment indicated via histochemical results that there is a definite and significant increase in NOS. Unfortunately, due to the overall condition of the animal, it was difficult to correlate these findings with those of the EFS. The animals that had undergone spinal cord transection

suffered alterations to many of the bodies' systems. It was not uncommon to find large pockets of pus encompassing the abdominal cavity, resulting in the fusion of the small and large intestine. Unusually large quantities of fluid in the abdominal cavity were also observed in several of the animals. Prostatic hyperplasia was observed as well as renal changes and possible renal failure (risk factor for impotence). It is quite possible that all of these conditions had deleterious effects on the animals' ability to respond to EFS.

Erectile dysfunction while not a life threatening disease is most definitely a life altering disease. The desire of ED suffers to find a satisfactory treatment option can be seen by the large response to the newly released oral treatment (sildenafil). Hopefully this is the beginning of a highly effective and successful treatment for those who suffer from ED, regardless of its etiology.