ANDROGENIC MAINTENANCE OF RAT PENILE ERECTION

by

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Submitted to the Faculty of the School of Graduate Studies of the

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Requirements for the Degree of Doctor of Philosophy
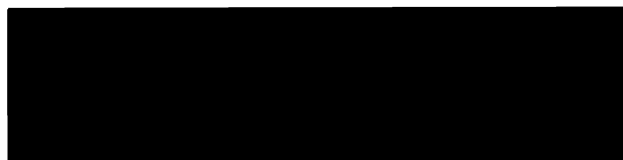
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1997.

Androgenic Maintenance of Rat Penile Erection

This dissertation is submitted by Christopher M. Reilly and has been approved by

an appointed committee of the faculty of the school of Graduate Studies on the Medical

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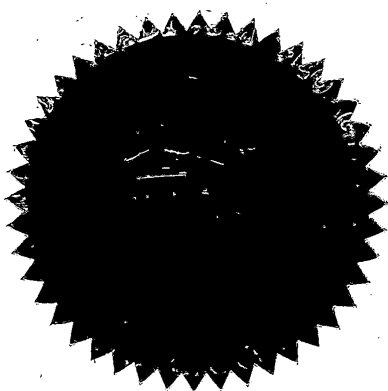
the degree of Doctor of Philosophy.

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DEDICATION

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PREFACE

In accordance with regulations of the Medical College of Georgia, School of Graduate

Studies, a student must present a dissertation as part of the graduation requirement of the

Doctor of Philosophy Degree. The Following dissertation includes three peer reviewed

accepted publications which are presented as published in their respective journals. Also

included is an Introduction, a preface to each manuscript, and a Summary and Conclusion

section which details how these data contribute to our understanding of how androgens

mediate erectile function.

CHAPTER 1

INTRODUCTION

Prevalence of Erectile Dysfunction: Erectile dysfunction is defined as the inability of a male to attain and maintain erection of the penis sufficient to permit satisfactory sexual intercourse. In 1992 a conference was convened by the National Institute of Health (NIH) to discuss the problem of erectile dysfunction. Following two days of presentations by researchers and clinicians and discussions by these experts in the field, the panel prepared a consensus statement. Among their findings, this conference concluded that erectile dysfunction is an important public health problem deserving increased support for basic science investigation (Anonymous 1992a). The NIH estimated that the number of U.S. men with erectile dysfunction is 10 - 20 million and the inclusion of individuals with partial erectile dysfunction increases the number to about 30 million. The problem afflicts 5% of men under the age of 40 increasing to 15 - 25% by the age of 65, and by the age of 75, nearly 50% of men suffer from erectile dysfunction (Anonymous 1992a). Similar conclusions were drawn from the Massachusetts Male Aging Study which concluded that impotence is a major health concern with multiple causes. The study concluded that the combined prevalence of erectile dysfunction (minimal, moderate, and complete) in men age 40 to 70 years old was 52% (Feldman et al, 1994).

Until recently, erectile dysfunction merited little scientific investigation because it was considered to be mainly psychogenic in origin (Lue 1993). Today, however, researchers have shown that organic origins account for the majority of incidences of

1

erectile failure (Speckens et al, 1993). Additionally, erectile dysfunction is considered to

be widely under reported, due to the stigma attached to a male being labeled as impotent.

Investigations into the causes of erectile dysfunction have also proven difficult, because of

problems in obtaining disease free intact cavernosal tissue from human subjects. Most of

the research using human cavernosal tissue has come from cadavers or biopsies of men

already suffering from erectile dysfunction. Animal models have proven useful in many

instances, but there are differences that exist between humans and animals making it

difficult to correlate findings in animals and men (Mills et al, 1996a).

Current treatment for erectile dysfunction can be divided into four basic categories

including the penile vacuum device, penile implants, vascular surgery, and

pharmacotherapy. The penile vacuum device, introduced commercially by Osbon in 1974,

creates a negative pressure around the penis, drawing blood into the corpora cavernosa.

Once erect, a restriction device is placed around the base of the penis to inhibit outflow

(Smith and Bodner 1993). Penile implants were developed in the 1970's. In the surgical

procedure to implant these devices, a rod shaped inflatable cylinder is implanted into each

corpus cavernosum with a fluid filled reservoir inserted into the scrotal pouch or abdomen.

In the inflatable types the fluid can be pumped from the reservoir into the cylinders inside

the corpora cavernosa resulting in rigidity of the penis (Gregoire 1992). Intracavernosal

injection was introduced by Brindly (1983) and Virag and Virag (1983). While Brindly

used an α adrenergic antagonist to decrease sympathetic tone and allow erection, Virag

and Virag injected a smooth muscle relaxant to cause erection. Later, a combination

therapy using both an α adrenergic antagonist with a phosphodiesterase inhibitor was

shown to be more effective in enhancing the erectile response (Zorgniotti 1988, Shenfeld et al, 1995). Although these treatments exist to manage patients with erectile dysfunction, there are no cures available, in part, due to a lack of understanding of the basic mechanisms involved in the erectile response. Normal erectile function requires the presence of androgens, although the mechanisms of how these steroids act to maintain the response is poorly understood.

Anatomy of the Human Penis: The penis consists primarily of three cylindrical structures, the paired corpora cavernosa arranged side by side on the dorsal portion of the penis and the corpus spongiosum on the ventral side (Figure 1). The corpus spongiosum surrounds the urethra and terminates as the glans penis. The paired corpora cavernosa are surrounded by a thick, relatively non-compliant collagenous structure called the tunica albuginea. These tunica converge medially to form the septum of the penis. In man and rats the midline septum is incomplete permitting blood to flow freely from one corpus cavernosum to the other. Although the tunica is attached to the deep penile fascia, sufficient space exists for the passage of blood vessels and nerves to allow blood flow into and out of the tissue as well as neuronal transmission. Surrounding the corpora cavernosa as well as the spongiosum is a thin strong layer of connective tissue (Buck's fascia). Blood flow to the cavernosal sinuses occurs via the penile artery which branches into four segments, the bulbar, urethreal, dorsal, and cavernous arteries. While the first three branches supply blood to the penis, only the cavernous artery enters the corpus cavernosum where it branches into smaller helicine arteries that empty into the cavernous

Figure 1. Cross section of the anatomical components of the human penis (adapted

from Lewis, 1995).

Skin

Superficial
dorsal.v

Deep
dorsal v

Dorsal
penile a

DORSAL

Tunica
albuginea

Circumflex v

Cavernosal a

Sinusoids
corpora-
cavernosa

Urethra

Corpus
spongiosum

VENTRAL
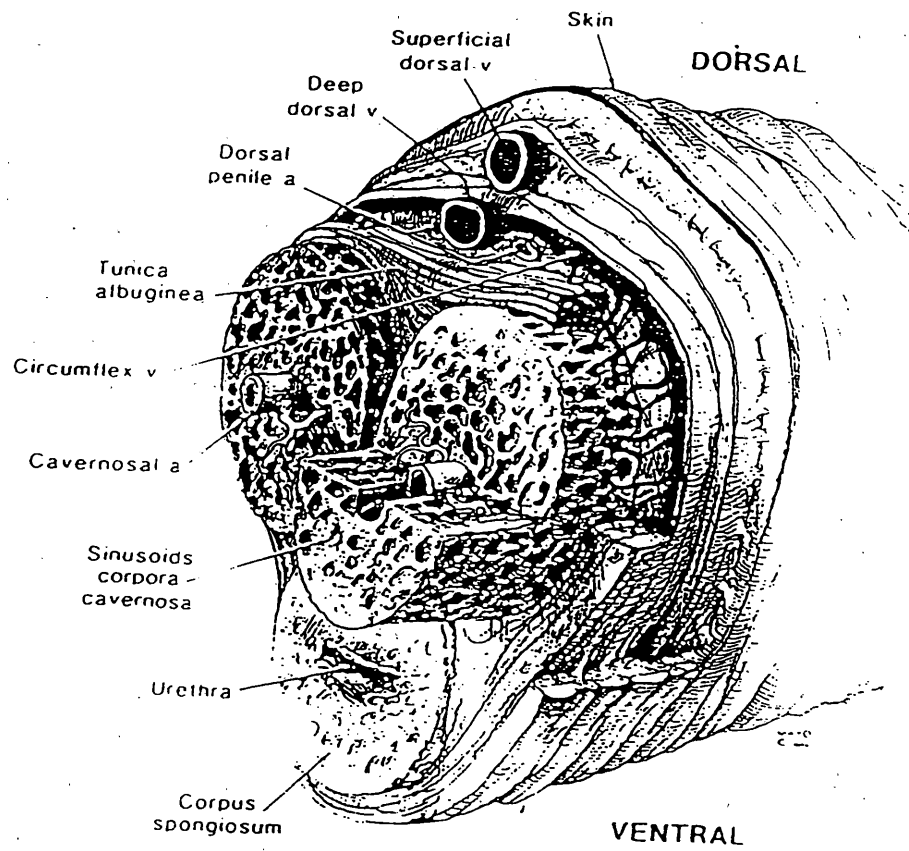
sinuses. The cavernous sinuses are comprised of a sponge-like meshwork that contains

smooth muscle, collagen, endothelial cells and extracellular matrix. Drainage from the

cavernosal tissue occurs via the emissary veins which cross the tunica albuginea emptying

into the circumflex veins and then into the deep dorsal vein (Lopez et al, 1991).

Physiology of Erectile Function: Penile erection (tumescence) and loss of erection

(detumescence) are regulated by relaxation and contraction of the corporal smooth muscle

respectively. Figure 2 illustrates the changes in the penis associated with erection. In the

detumescent state, vascular smooth muscle of the corpus is tonically contracted, allowing

only minimal blood flow into the tissue for nutritional purposes (1 - 4 ml/min/100 g of

tissue) (Wagner 1981). Erection is controlled by the autonomic nervous system and

involves three complementary and simultaneous processes: First, a decrease in the release

of norepinephrine from the adrenergic nerves innervating the penis lowers the sympathetic

tone in the cavernosal smooth muscle. Second, discharge of non-adrenergic

non-cholinergic (NANC) neurotransmitters, including nitric oxide (NO), increase the

second messengers (cGMP and possibly cAMP) resulting in relaxation of smooth muscles

within the corpus cavernosum, both of which produce a large increase of blood flow and

subsequent cavernosal sinus engorgement. Third, as the sinuses fill with blood they

compress the subtunical veins against the relatively inelastic tunica albuginea inhibiting

venous outflow (veno-occlusion). The net result of these three processes is rigidity of the

penis. After ejaculation there is a re-establishment of sympathetic tone, a decrease in the

release of vasodilatory neurotransmitters, and the inactivation of second messengers by

Figure 2. Top: Wedge section of the intracavernosal tissue of the human penis in the flaccid state. Note the blood freely drains through the cavernosal tissue via the non compressed emissary veins. Bottom: Wedge resection of the intracavernosal tissue of a human penis in the erect state (adapted from Lewis, 1995). Note that the corpus cavernosum has expanded inhibiting the outflow of blood though the emissary veins.

Emmissary v

Circumflex v

Corpus
cavernosum

Cavernosal a

RELAXED

Emmissary v

Circumflex v

Corpus
cavernosum

Cavernosal a
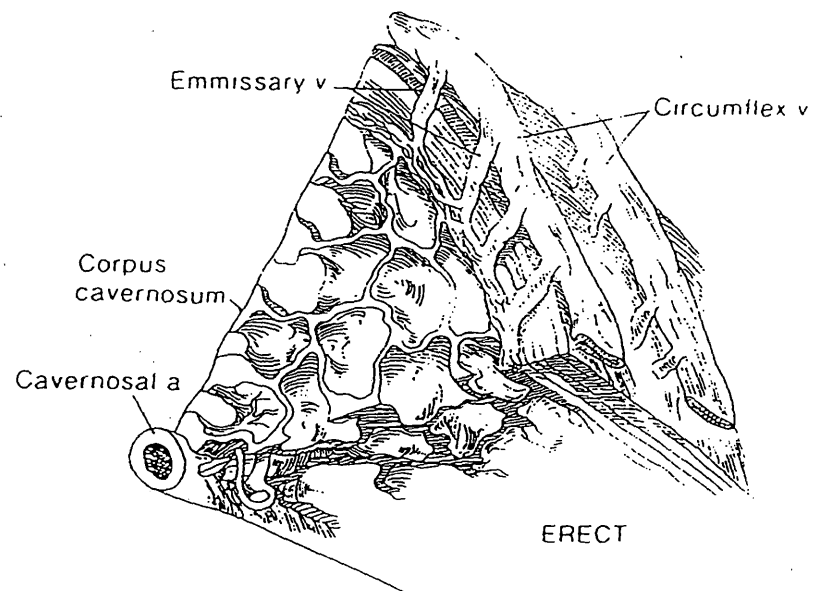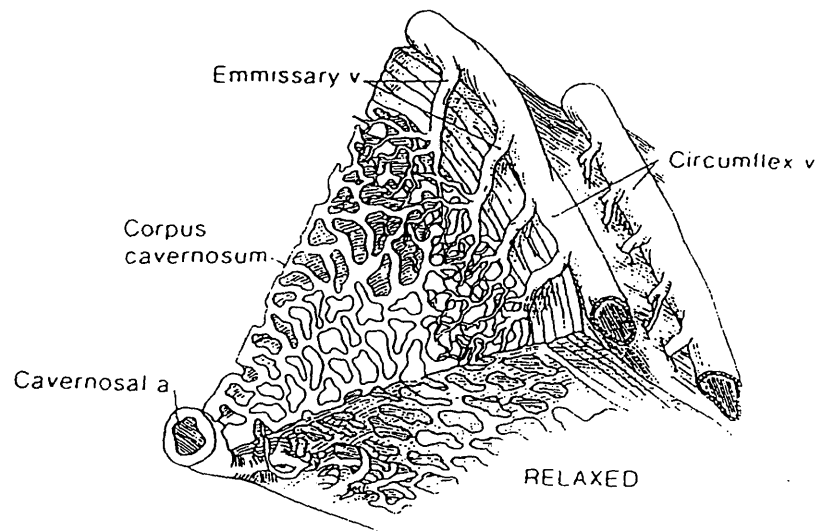
ERECT

phosphodiesterase enzymes. Together, these events result in the inactivation of the veno-occlusive mechanism permitting venous outflow and draining of blood from the penis.

Pathologies of Erectile Dysfunction: Erectile dysfunction can be broadly divided into organic and psychogenic causes with nearly 80% being classed as organic (Heller and Gleich 1988), although many patients suffer mixed etiologies (Sergravers and Sergraves 1991). Psychogenic factors resulting in a loss of erectile function are often associated with anxiety and include, excessive sympathetic outflow and elevated blood catecholamine levels, both of which may increase smooth muscle tone and prevent smooth muscle relaxation and erection (Korenman, 1995). Organic dysfunction can be further divided into several different subcategories including venogenic, arteriogenic, neurologic, traumatic, and endocrinologic. Venogenic and arteriogenic causes often coexist and have been determined to account for as many as 50% of men suffering organic erectile dysfunction (Virag et al, 1985; Krane et al, 1989). Cavernosal arterial insufficiency, either atherosclerotic or traumatic in origin can decrease perfusion pressure and limit arterial inflow to the cavernous spaces (Michal, 1982). The most common cause of arterial insufficiency is believed to be arteriosclerotic vascular disease (Azadzoi and Goldstein 1992). Medications commonly used in treatment of hypertension including diuretics, β adrenergic blockers, arterial vasodilators, and calcium antagonists may also result in erectile dysfunction (Berger, 1994). In men with adequate inflow, outflow through the subtunical veins is excessive in venogenic impotence resulting in inadequate penile rigidity

(Aboseif et al, 1990). Venogenic causes of impotence include the development of excessively large venous channels through the tunica albuginea. Furthermore, degeneration of the tunica albuginea results in inadequate compression of the subtunical veins. Injury or disease of the cavernosal smooth muscle which prevents sufficient expansion of the sinuses may also result in veno-occlusion failure. Additionally, venous shunts between the cavernosum and the spongiosum, due to damage or congenital defects, allow excess drainage (Wespes and Schulman 1993).

The penis is innervated by both parasympathetic and sympathetic nerve fibers in addition to somatic fibers so that damage to either somatic or autonomic fibers can result in erectile dysfunction. In the parasympathetic nervous system, the pelvic and the pudendal nerves arise from the S-2 to S-4 segments of the spinal cord. While the pudendal nerves innervate the penis directly, the pelvic nerve joins fibers from the hypogastric nerves (sympathetic T-11 to L-2) to form the pelvic plexus. In this plexus the sympathetic fibers merge with the parasympathetic fibers and exit as part of the cavernous nerves. The pelvic plexus lies very near the posterior lateral surface of the prostate and fibers from it innervate the rectum, bladder, prostate, seminal vesicles as well as the penis. The cavernous nerves arising from the pelvic plexus supply neuronal input to the corpus cavernosum, corpus spongiosum, and the glans penis. Within the corpora cavernosa the cavernous nerves supply the cavernous vein and terminal branches of the nerve innervate the urethra, dorsal, and deep arteries of the penis (Berger et al, 1994). Numerous diseases are associated with both somatic and autonomic peripheral neuropathy and can cause

impotence. These include peripheral, hereditary, as well as toxic neuropathies (Berger et al, 1994).

Endocrine causes of impotence include specific diseases that lead to hypogonadism and erectile failure and can be classified as either hypergonadotropic (testicular failure) or hypogonadotropic (pituitary-hypothalamic) disorders (Glass and Vigersky 1991). In both instances the result is a decrease in testosterone production. (The critical role of testosterone in the maintenance of erectile function is discussed extensively in Chapters 2, 3, and 4, below). Other endocrine pathologies can also lead to diminished erectile function. These conditions include diabetes mellitus, thyroid dysfunction, and adrenal disease. Some diabetics have diminished testosterone production (Barrett-Connor 1992), peripheral neuropathy (Bemelmans et al, 1994), and arterial insufficiency (Wang et al, 1993). Erectile dysfunction has been associated with both hyperthyroidism and hypothyroidism. In patients with hyperthyroidism, serum levels of testosterone-binding globulin (TeBG) are often elevated and luteinizing hormone (LH) and estradiol levels are decreased (Chopra and Tulchinsky 1974). Individuals with hypothyroidism often show decreased testosterone levels, possibly due to the decreased production of TeBG resulting in increased clearance of free testosterone (Kidd et al, 1979). Hypothyroid individuals commonly have elevated prolactin secretion which may also contribute to the diminished erectile function (Snyder et al, 1973). Hypogonadism is often seen in patients with chronic liver disease particularly in alcoholics, in part, due to the direct toxic effects that alcohol has on Leydig cells (Van Thiel et al, 1974). Patients with adrenal disease, particularly Cushing's disease, may exhibit decreased libido. Patients suffering from this

disease may have both testicular failure and hypothalamic-pituitary disorders (Luton et al, 1977).

Hypothalmic-Pituitary-Gonadal-Axis: Serum testosterone levels are regulated by the hypothalamic-pituitary-gonadal axis (Figure 3). Gonadotropin releasing hormone (GnRH) is secreted in a pulsatile pattern by the hypothalamus and binds to specific high affinity receptors in anterior pituitary cells which respond by releasing LH and follicle stimulating hormone (FSH). While FSH is responsible for sperm production by binding to its receptors on Sertoli cells in the testes (Griffin 1996), LH binds to receptors on Leydig cells in the testes and this binding increases cholesterol transport into the testes, cholesterol side chain cleavage (CSCC) activity, and *de novo* testosterone synthesis (Griffin 1996). As LH acts to increase testosterone production, this androgen as well as its aromatized metabolites, estradiol, and the 5 α reduced metabolite, dihydrotestosterone (DHT) exert a negative feedback on the pituitary/hypothalmus to decrease the production of LH (Lin and Clark 1996). DHT has been shown to decrease the amplitude of the LH release, whereas testosterone decreases GnRH pulse frequency from the hypothalamus. Estradiol dampens the pulsatile secretion of LH by the anterior pituitary as well as decreasing the frequency of GnRH pulses (Lin and Clark 1996).

Availability of Testosterone: Under normal circumstances about 60 % of testosterone in plasma is bound to albumin and 37 % is bound to TeBG (Lin and Clark 1996). The binding of testosterone to these plasma proteins has traditionally been thought to prevent

Figure 3. The hypothalamic-pituitary-gonadal axis in the male.

CNS-Central nervous system

GnRH-Gonadotropin releasing hormone

LH-Luteinizing hormone

FSH-Follicle stimulating hormone

T-Testosterone

E-Estradiol

DHT-Dihydrotestosterone
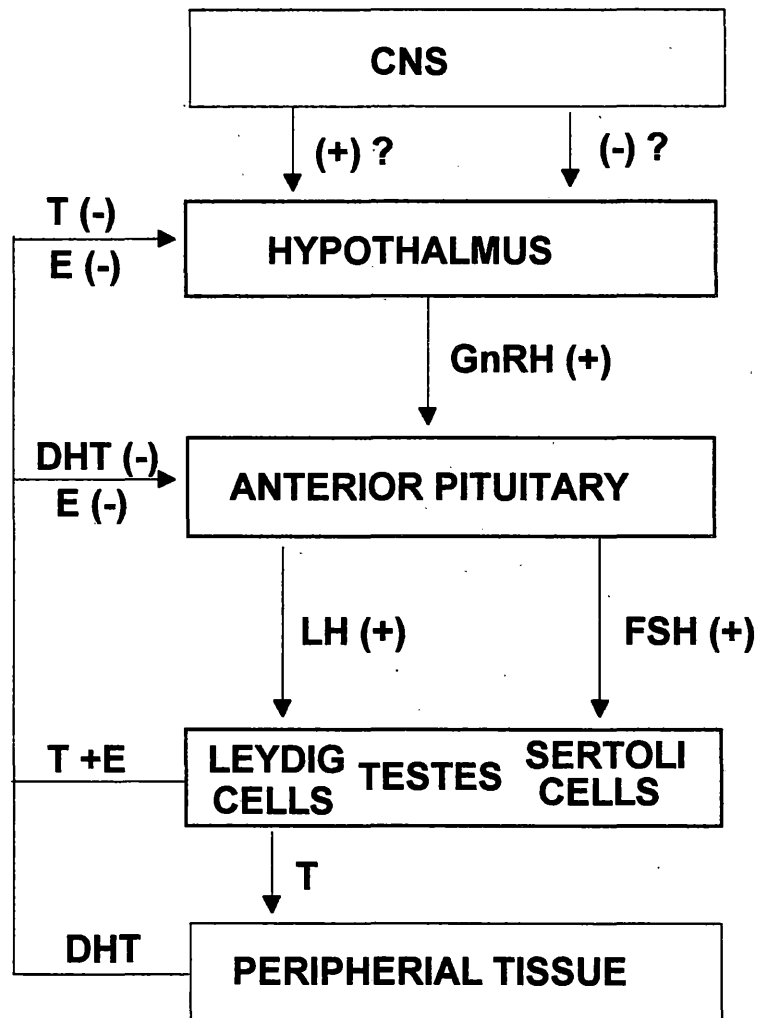
its biological activities allowing only 1 - 3% of unbound testosterone to exert a biological influence on its target tissues. More recent reports suggest, however, that since the binding of testosterone to albumin has a relatively high dissociation constant, possibly 50% of the albumin bound testosterone is biologically active testosterone (Lin and Clark 1996).

Testosterone is synthesized primarily by the Leydig cells located in the testes, although in some species including humans the adrenal glands, fat tissue, and skin contain small amounts of the enzymes necessary for testosterone synthesis (Lin and Clark 1996). The main substrate for the synthesis of testosterone is cholesterol produced either *de novo* from acetate or delivered to the cells by plasma lipoproteins. The synthesis of testosterone from cholesterol is shown in Figure 4 involving five enzymes, all of which have been identified in Leydig cells. The rate limiting step in testosterone synthesis is the activity of CSCC, under the influence of LH (Griffin 1996).

Testosterone Treatment for Erectile Dysfunction: Although testosterone replacement has restored erectile function in some hypogonadal patients (McClure 1988; Clark et al, 1984), many clinicians question the importance of androgens in the maintenance of erectile function in men. This doubt can be attributed to three observations: First, in some cases, hypogonadal men continue to experience normal erectile function (Bancroft and Wu 1983; Shah et al, 1988). Second, treatment of impotent patients with androgens does not always yield positive results (Leonard et al, 1989), and third, castration often does not lead to an immediate loss of potency. While a majority of castrated men eventually become impotent,

Figure 4. Steroidogenic pathway for the synthesis of testosterone.

Cholesterol

| CSSC

Pregnenolone ⟶ Progesterone

17-Hydroxylase

17-OH-Progesterone ⟶ 17-OH-hydroxyprogesterone

17-20 Lyase

Dehydroepiandrosterone ⟶ Androstenedione

17 β HSD

Androstenediol ⟶ Testosterone

3 β Hydroxysteroid dehydrogenase

5 α Reductase   Aromatase

Dihydrotestosterone   Estradiol
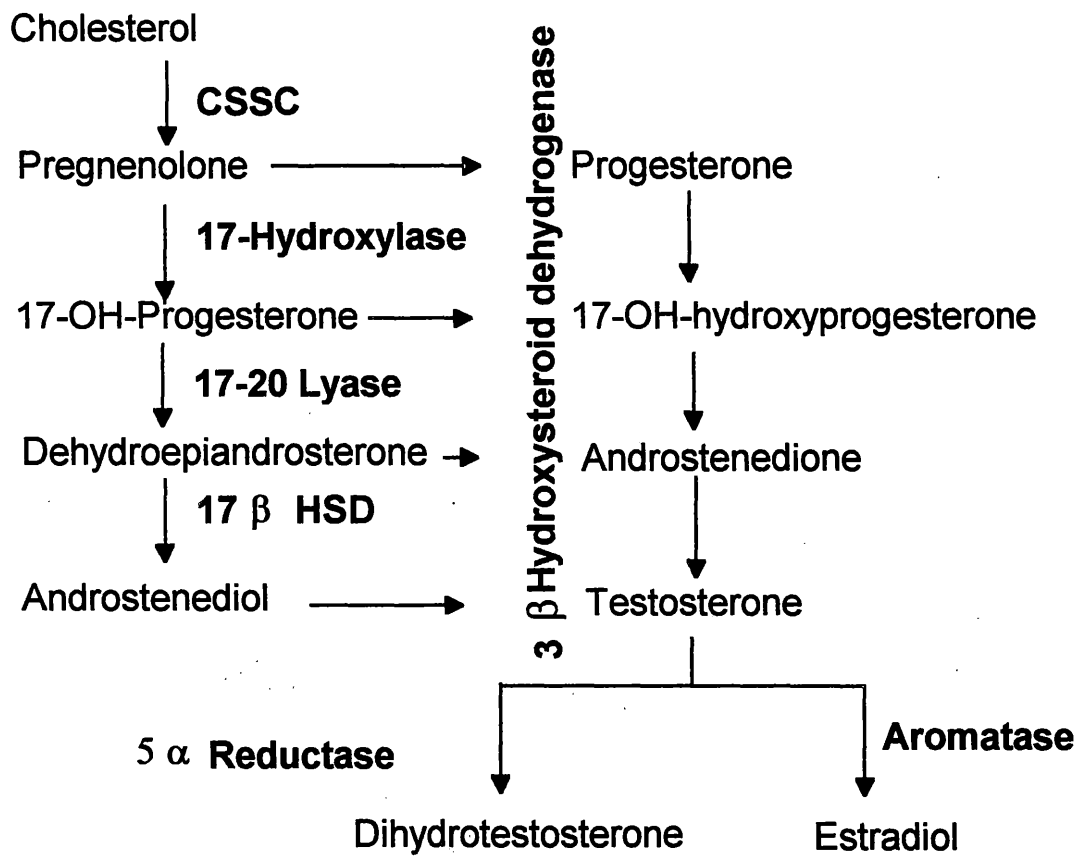
a small percentage of individuals retain erectile function (Ellis and Grayhack 1963;

McCullagh and Renshaw 1934). Although these observations support the idea of

androgens being inconsequential for erectile function in the adult, it is important to note

that the human adrenal produces substantial amounts of dihydroepiandrosterone (DHEA)

and androstenedione. The conversion of these steroids to testosterone and

dehydrotestosterone (DHT) occurs in peripheral tissues other than the testes (Labrie et al,

1988), although in reduced quantities. In some men conversion may be sufficient to

maintain the capacity for erection. Greenstein et al, (1995) reported differences in the

erectile function of men undergoing surgical castration vs. chemical castration. In

surgically orchidectomized men, 25% responded to erotic visual stimulation and had free

testosterone levels of 1.125 $\pm$ 0.036 pg/ml which was significantly higher than men who

failed to respond to stimulation and had free testosterone levels of 0.628 $\pm$ 0.098 pg/ml.

In men that had undergone chemical castration by being treated with DES, flutamide, or

GnRH analogs, all failed to respond to erotic stimulation and had free testosterone levels

of 0.620 $\pm$ 0.021 pg/ml. The idea that the erectile response can be maintained with

relatively low amounts of androgen is supported by other investigators who suppressed

endogenous secretion of testosterone in normal healthy men by the use of a gonadotropin

releasing hormone (GnRH) agonist. The researchers then assessed sexual activity and

erectile response during replacement with exogenous testosterone (Buena et al, 1993).

The authors report that even though the androgen replacement only raised the blood levels

of testosterone to the very low end of the normal range, erectile activity and libido were

completely maintained. These findings which showed that the erectile response can be

maintained with low amounts of androgen produced by the adrenal glands, may explain

why some hypogonadal men have normal erections and nocturnal penile tumescence

episodes (NPT) (Buena et al, 1993).

Administration of antiandrogens such as cyproterone acetate and flutamide, abolish

erectile function in many instances (Rousseau et al, 1988), although some men retain

potency (Fleshner and Trachtenberg 1993). Erectile dysfunction and low blood

testosterone levels are also problems with men undergoing renal dialysis (Coppola and

Cuomo 1990). Prolactin secreting tumors of the pituitary often decrease serum

testosterone levels and lead to erectile dysfunction (Carter et al, 1978), although in some

patients, serum testosterone levels remain normal (Carani et al, 1995). While

bromocriptine administration decreases prolactin secretion, testosterone production does

not always return to normal and these individuals may require exogenous testosterone

administration to return the full erectile response (Moralas et al, 1994). In potent men

with normal testosterone levels, it has been reported that exogenous androgen

administration increased the rigidity of their erections, suggesting that testosterone acts on

the cavernosal arterioles to control blood flow into the sinuses (Cunningham et al, 1990).

As males age the amount of testosterone present in the blood decreases

(Korenman et al, 1990; Morley and Kaiser 1989; Morley et al, 1987), and several authors

have reported that episodes of NPT and quality of spontaneous erections are diminished in

older men and hypogonadal men (Rowland et al, 1993). Furthermore, treatment of older

men with testosterone or gonadotropin improves the quality of erections and NPT

frequencies (Cunningham et al, 1990). In a literature review by Mulligan and Schmitt

(1993) it was stated that testosterone replacement is important for sleep related erections

but evidence was inconclusive as to the importance of androgens in the maintenance of

the erectile response to fantasy or visual erotic stimulation. The authors also suggest the

possibility of an androgen independent and androgen dependent mechanism in erectile

function, with NPT episodes requiring the presence of testosterone, while the erectile

response to visual sexual stimulation is independent of testosterone. Clearly, these

studies and others (Schiavi et al, 1993; Carani et al, 1992; Carani et al, 1990; Anderson et

al, 1992; Burris et al, 1992; Cunningham et al, 1990; Carey et al, 1988) demonstrate that

testosterone mediates erectile function, but questions remain as to mechanism of action

and the degree to which testosterone influences the erectile process.

Androgenic Maintenance of Erectile Function in Rats: The rat serves as a useful

model for the study of penile erection because the mechanisms involved in rats are similar

to those of humans, although there are some differences (Fernandez et al, 1991). Figure 5

is a schematic diagram of a rat penis. Unlike the human penis where the subtunical veins

become compressed beneath the tunica albuginea inhibiting outflow, in rats the cavernous

vein branches into a series of parallel channels that rejoin to form the crural vein crossing

the tunica albuginea. It is thought that these channels become compressed as the

cavernous sinuses fill thus inhibiting outflow. The corpora cavernosa and corpus

spongiosum in the rat are independent erectile tissues, each with its own arterial and

venous vessels. Furthermore, rats possess an os penis, a cartilaginous structure in the

distal portion of the penis between the corpus cavernosum and the glans penis. Other than

Figure 5. Schematic representation of the vasculature of the rat penis (adapted from

Fernandez et al, 1991).

DEEP DORSAL VEIN

CAVERNOUS VEIN

CRURAL VEIN

DEEP ARTERY
OF PENIS

URETHRA

ARTERY OF BULB
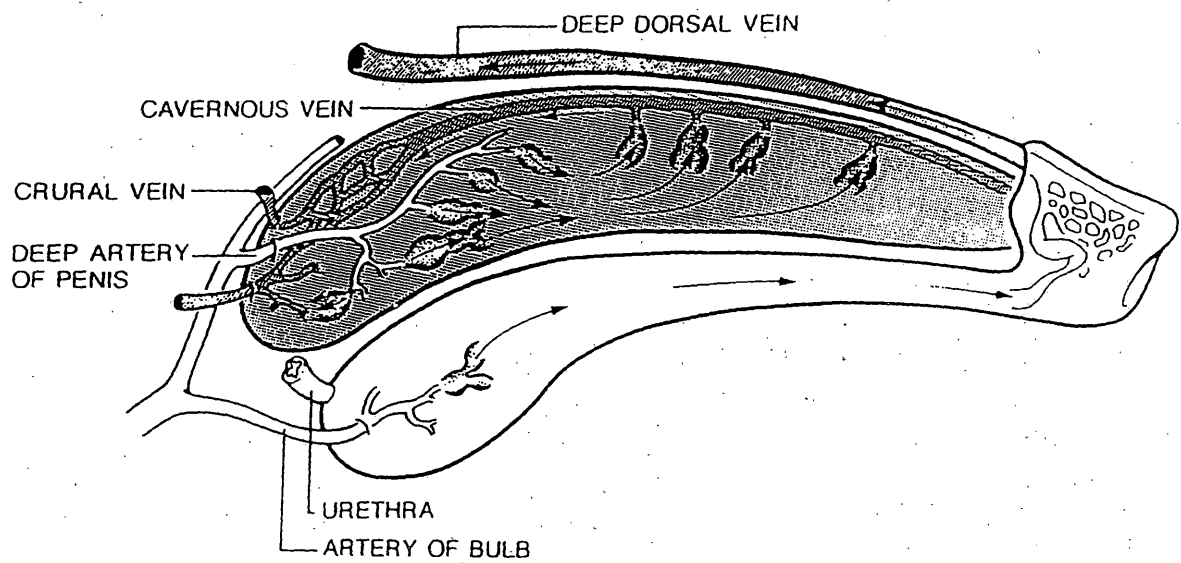
these differences, rats are considered an appropriate model for the study of erectile

function. They are especially well suited for investigating the effects of complete androgen

withdrawal on erectile function since the rat adrenals lacks 17 β hydroxylase activity and

therefore, produces no androgenic steroids (Labrie et al, 1988). Thus, by one week after

castration, the plasma levels of DHEA, androstenedione, DHT and testosterone have

fallen to low levels, and erectile function is diminished (Labrie et al, 1988; Giuliano et al,

1993b; Mills et al, 1992). By using castrated rats, studies from several laboratories have

shown that androgen replacement restores the erectile response. Using castrated animals

with spinal cord transection, Hart (1967) demonstrated a decrease in the erectile response

(*ex copula*) but the response was restored with androgen treatment. The facilitory role of

androgens on erectile function has been verified in subsequent studies (Keast,1992).

Others have shown that an inhibitor of 5 α reductase (17- β testosterone carboxylic acid),

when given with testosterone, suppresses erectile function but does not diminish

copulatory behavior (Bradshaw et al, 1981). DHT is as effective as testosterone in

restoring the erectile response in castrated animals but treatment with the 5 α reductase

inhibitor (finasteride) significantly decreased the responsiveness of castrated animals

treated with testosterone (Lugg et al, 1995). Blockage of testosterone action with the

anti-androgen flutamide resulted in abolition of both copulatory and erectile

responsiveness (Gray et al, 1980). Furthermore, infusion of testosterone in castrated

animals did not restore erectile function immediately; androgen replacement required 24 -

48 hours for the return of the erectile response (Sachs and Leipheimer 1988). Mills et al.

(1994) have demonstrated that castrate animals require an increased rate of perfusion of

saline into the cavernous sinuses to maintain high levels of intracavernosal pressure suggesting androgens also act to maintain the veno-occlusive mechanism. Taken together these studies demonstrate that in the rat, erectile function is regulated to a significant degree by androgens acting on penile tissue. This project was, therefore, undertaken to investigate the sites of testosterone action in the maintenance of erectile function.

Statement of the Hypothesis: Androgens act to maintain the erectile response in the rat penis by regulating multiple pathways involved in the erectile response.

Explanation of Hypothesis: Stimulation of cAMP or cGMP pathways result in erection in the human penis. These pathways are activated by several neuropeptides and neurotransmitters including acetylcholine, vasoactive intestinal peptide, nitric oxide, and calcitonin gene related peptide. Additionally, other neuropeptides and neurotransmitters, such as norepinephrine and endothelin -1 have been identified to be detumescence factors. Since androgens are required for the maintenance of erectile function, experiments were performed to determine where androgens act to maintain erectile function.

CHAPTER 2

Specific aim 1: Determine if androgens regulate the abundance of neuronal nitric oxide synthase (nNOS) mRNA in the corpus cavernosum in rats.

PREFACE

Role of Nitric Oxide (NO) in the Erectile Response in the Penis: It is generally accepted that NO, a neurotransmitter which originates in nonadrenergic noncholinergic (NANC) nerve fibers and endothelial cells, is involved in vasodilation of the cavernosal vasculature. Several authors have demonstrated that inhibitors of NO abolish erectile function (Holmquist et al, 1991; Pickard et al, 1991). Others have demonstrated tumescence with NO releasers (Kim et al, 1991; Ignarro et al, 1990; Burnett et al, 1995; Burnett et al, 1992). NO, released from the NANC nerve terminals in the corpora cavernosa binds to the heme moiety of soluble guanyl cyclase and this binding activates the conversion of GTP to cGMP in smooth muscle target tissue (Waldman and Murad 1987). The increase in cGMP modulates other intracellular processes leading to decreased intracellular Ca^{2+} and smooth muscle relaxation. These intracellular processes activated by cGMP include:

1. Interference with the intracellular increase of Ca^{2+} necessary for activation of myosin light chain kinase (McDonald and Murad 1996).

2. Phosphorylation and activation of Ca^{2+}-ATPase associated with membrane transport (Mayer, 1994).

3. Inactivation of phosphodiesterase enzymes that convert cAMP and cGMP to their

   inactive 5' nucleotides (Lugnier and Komas 1993).

4. Inactivation of phospholipase C, the enzyme responsible for the formation of

   inositol 1,4,5 triphosphate (IP_3), a second messenger involved in raising Ca^{2+}

   levels (McDonald and Murad 1996).

The synthesis of NO occurs as the principal product of the enzymatic conversion of

L-arginine to L-citrulline by the enzyme nitric oxide synthase (NOS). NOS has been

identified in many tissues including the penis. The precise localizations of NOS in the

vasculature and innervation of the penis have been established using immunohistochemical

and enzymatic methods. In the rat (Burnett et al, 1992), dog (Burnett et al, 1992), bull

(Sheng et al, 1992) and man (Burnett et al, 1993), neuronal NOS (nNOS) activity has

been identified in the pelvic plexus, the cavernous nerves, the terminal nerve endings in the

cavernous tissue, the dorsal penile nerves, and the nerve plexus of the dorsal and

cavernosal arteries and their branches, the helicine arteries. Bilateral cavernous nerve

transection in rats abolished immunohistochemical staining in NOS-containing penile

neurons while staining persisted in the vascular endothelium (Burnett et al, 1992). It was

inferred from these data that NO acts as a post-ganglionic neurotransmitter and is released

through efferent neuronal pathways in response to stimulation. The staining that persisted

in the vascular endothelium was determined to be an endothelial isoform (eNOS) of the

enzyme, biochemically distinct from the neuronal isoform (Lamas et al, 1992). The eNOS

form has been shown to be released under acetylcholine stimulation (Saenz de Tejada et

al, 1988). However, since the removal of endothelial cells from corporal tissue in *in vivo*

experiments did not eliminate vasodilation, it can be concluded that eNOS plays only a minor or auxiliary role in erectile function (Bookstein et al, 1990). Several authors have speculated that androgens maintain erectile function by regulating levels of NO. These reports include Mills et al. (1992) who demonstrated a failure of penile vascular tissue to respond to nitroglycerine infusion in castrate-untreated (CASTRATE) but not castrate-testosterone treated (TESTO) animals and equated this finding to the androgenic maintenance of nNOS activity. Garban and coworkers (1995a,b) demonstrated by arginine-citrulline conversion, that nNOS activity is decreased in castrate animals but not in castrate animals given DHT. Chamness et al. (1995) demonstrated that castrate animals possess about one half the amount of nNOS protein that is found in testosterone treated animals. Using NADPH diaphorase staining to localize NOS activity, Zvara et al. (1995) demonstrated that NOS enzyme in the cavernosal nerve fibers was decreased by more than 50% in castrated animals, but was restored with testosterone treatment. Although these studies correlate NOS activity with testosterone levels, there have been no published reports that testosterone enhances the expression of the nNOS gene, or in any other way increases the nNOS mRNA levels in the rat penis.

The following is the text of a manuscript entitled "Androgenic Regulation of NO Availability in Rat Penile Erection." This manuscript shows the importance of NO in the erectile response and demonstrates that testosterone enhances the levels of available nNOS mRNA in the corpus cavernosum. The authors of this manuscript are Christopher M. Reilly, Pedro Zamorano, Vivienne S. Stopper, and Thomas M. Mills, and was published in the *Journal of Andrology* 1997; 18:110-115.

ABSTRACT

Prior studies from this laboratory, using untreated-castrated rats (CASTRATE) and testosterone-treated castrated rats (TESTO), have shown that the magnitude of the intracavernosal pressure increase during erection is androgen dependent. Studies from this and other laboratories have also presented evidence suggesting that penile erection is mediated principally by nitric oxide (NO). The present report was designed to confirm that androgens maintain the availability of cavernosal NO, and to determine if this androgenic action is exerted at the genomic level modulating the expression of the neuronal form of nitric oxide synthase (nNOS) gene. The results showed that administration of supplemental L-arginine failed to augment the erectile response in either group, suggesting that substrate availability is not a cause of the reduced response in CASTRATE animals. Inhibition of NO synthesis with a nitro-arginine competitive inhibitor of NOS resulted in strong inhibition of erection in both TESTO and CASTRATE rats. When given in conjunction with ganglionic stimulation to induce erection, the NO releasing drug, sodium nitroprusside (SNP), increased intracavernosal pressure in CASTRATE but not in TESTO rats suggesting a deficiency of the available NO in CASTRATE animals. Finally, reverse transcription-polymerase chain reaction (RT-PCR) demonstrated that mRNA levels for the enzyme nNOS in the penis were greater in TESTO animals than in CASTRATE rats. These results support the hypothesis that androgens mediate the erectile response in the rat penis by stimulating the expression of the neuronal isoform of nitric oxide synthase, thus maintaining an adequate supply of NO.

KEY WORDS: penile erection, rats, testosterone, nitric oxide

INTRODUCTION

Studies from this, and other laboratories, have demonstrated that erection in the rat is androgen dependent and is mediated by nitric oxide (Andersson and Holmquist 1994; (Burnett 1995). Results from several groups support the hypothesis that the primary action of androgens is to stimulate the synthesis of NO (Zvara et al, 1995; Garban et al, 1995; Mills et al, 1992; Mills et al, 1994; Chamness et al, 1995), specifically the neuronal isoform (nNOS), which has been identified in nerve fibers innervating blood vessels and the corpus cavernosum of the penis (Burnett et al, 1992; Keast 1992; Vizzard et al, 1994). These findings included the observation that the amount of nitric oxide synthase (NOS) enzyme protein was reduced in castrated animals as compared to animals with normal blood levels of testosterone (Chamness et al, 1995). However, none of these prior studies establish the mechanism by which androgens stimulate an increase in NO. The present study includes experiments which were designed to confirm that NO is a principal neurotransmitter in the erectile response of the rat penis and to investigate two mechanisms by which androgens could elevate NO levels. These two mechanisms include an androgenic action to increase the availability of L-arginine substrate for the NOS reaction and an action of androgens to increase the expression of the nNOS gene.

MATERIALS AND METHODS

Animals: Male Holtzman rats (Harlan), 100- 240 days of age, weighing 400-600 gms were used in these studies. Each animal was castrated under ether anesthesia and a pellet of testosterone (50% testosterone: 50% cholesterol - group designation: TESTO) or cholesterol pellet alone (group designation: CASTRATE) was implanted subcutaneously. Animals were castrated and implanted with pellets 6 - 8 days before measurements were made. At the end of each experiment, blood was collected from the carotid artery for measurement of circulating levels of testosterone by radioimmunoassay as previously described (Melner and Abney 1980). Animals were maintained in an AAACAC accredited laboratory facility with animal use protocols and justifications approved by the Committee for Animal Use in Research and Education at the Medical College of Georgia.

Measurement of Intracavernosal Pressure: The procedure used to induce erection and measure intracavernosal pressure has been previously published (Mills et al, 1994; Mills et al, 1992). Briefly, the animals were anesthetized with ketamine (87 mg/kg) and xylazine (13 mg/kg) by intramuscular injections and supplemented with additional anesthesia as needed. Standard methods were used to cannulate the left carotid artery for continuously monitoring mean arterial blood pressure (MAP). The abdominal cavity was opened, the viscera retracted and wrapped in warmed saline soaked sponges and cellophane wrap to prevent drying. The right major pelvic ganglion was exposed by clearing the overlying fascia. The shaft of the penis was dissected free of skin and fascia to expose the paired corpora cavernosa distal to the crura. The right corpus cavernosum was cannulated with a

30 gauge needle attached to PE 200 tubing to monitor intracavernosal pressure. A second cannula was inserted into the left corpus cavernosum for intracavernosal drug delivery. Stainless steel bipolar electrodes were then positioned on the major pelvic ganglion for ganglionic stimulation to induce erection. Intracavernosal pressure (CCP), mean arterial blood pressure (MAP) and duration, frequency and amplitude of the ganglionic stimulation were continuously recorded on a polygraph recorder. Results are expressed as the ratio of intracavernosal pressure (CCP) to the mean arterial pressure (MAP). According to this method of expression, a ratio of 0 would indicate that there was no measurable intracavernosal pressure while the CCP/MAP ratio would be 1 if the CCP and MAP were equal. Stimulatory voltage was varied from 1 to 6 volts (5 msec duration, 12 Hz frequency) and the CCP/MAP ratio at each voltage compared to determine the threshold voltage and the minimum voltage required for the maximal response. A voltage of 5 or 6 volts was used in subsequent aspects of each experiment. The duration of each ganglionic stimulation was 1 minute with a 1 minute rest period between subsequent stimulations. Pressure transducers were calibrated with a mercury manometer prior to each use.

Intracavernosal Infusion of L-Arginine: To determine if substrate availability was limiting the erectile response in castrated animals, the response was measured after direct administration of L-arginine into the corpus cavernosum. Following a control measurement of erection, a single injection of L-arginine (25 µg/kg in 10 µl saline) was made into the right cavernous sinus and the erectile response was measured 5 minutes

later. Additional L-arginine was given by continuous intra-aortic infusion (50 µg/kg/min infused at 20 µl/min) with the erectile response measured after 5, 10, and 15 minutes of infusion.

Drug administration: Intracavernosal injections were made of two drugs. Sodium nitroprusside (SNP): Eight µg/kg body weight in 1 µl saline was injected 3 minutes before ganglionic stimulation to induce erection. This drug acts as a vasodilator via the release of NO (Martinez-Pineiro et al, 1993; Feelisch 1991). N-nitro-L-arginine (L-NNA): Two hundred µg/kg body weight in 5 µl saline were injected 10 minutes before ganglionic stimulation. L-NNA is a competitive inhibitor of nitric oxide synthase (Ward and Angus 1993).

Analysis of the effects of androgen treatment on cavernosal nNOS gene expression

Preparation of cavernosal mRNA: Following death of the animal by cervical dislocation, the entire penis was removed from TESTO and CASTRATE rats and the proximal shaft and the crural regions collected. The distal portion of the penis containing the os penis was discarded and the tissue was immediately placed in RNAzol (2 ml/100 mg tissue wet weight) and RNA isolated according to the protocol supplied with the RNAzol (Biotecx Laboratories, Houston TX). The concentration of RNA in each sample was determined by measurement of the absorbance at $\lambda = 260$ and 280 nM and diluted with 75% ethanol to a final concentration of 1µg/µl for storage at -70 °C.

Reverse transcription reaction: The RNA in each sample was precipitated by addition of one-tenth volume of 2 M sodium acetate (pH 4.7) followed by a 15 min centrifugation at 12,000 X g. The RNA was re-suspended in DEPC treated water and subjected to the reverse transcriptase reaction to synthesize cDNA using M-MLV Reverse Transcriptase (Promega, Madison WI). In this procedure 0.8 µg of total RNA was primed with 0.5 µg oligo (dT) 12-18 and incubated for 10 minutes at 70 °C in a 10 µl volume and then frozen on ice. Next, for each sample, 1 X M-MLV buffer (50 mM Tris-HCl, pH 8.3, 75 mM KCl, 3 mM MgCl$_2$, 10 mM DTT), 0.5 mM dNTP mix, and 200 units of M-MLV reverse-transcriptase in a volume of 20 µl were combined and incubated for 40 minutes at 40 °C. After incubation, the samples were stored at -20 °C.

Polymerase chain reaction: The oligonucleotide primers for nNOS had the following sequence: upper primer (sense strand) 5'- ACC TGA AGA GCA CAC TGG AAA C- 3' and lower primer (antisense strand) 5'- GAT GGC CGA CCT GAG ATT C- 3' that amplified a 428 bp fragment for the nNOS sequence. For an internal standard, cyclophilin mRNA was also amplified. The primers for the cyclophilin gene had the following sequence: upper primer 5'-TGT TCT TCG ACA TCA CGG C- 3' and lower primer 5'-TTA TGG CGT GTG AAG TCA CC- 3' amplifying a transcript of 216 bp. In preliminary cycling experiments the expression of cyclophilin was found to reach the plateau phase which was not proportional to that of the nNOS gene. To correct for the difference in the original mRNA levels the "primer dropping" method (Wong et al, 1994) was employed. In this method, 1.0 µl of the cDNA product was added to a final volume of 49 µl

containing 1.5 mM $MgCl_2$, 1X PCR buffer (20 mM Tris-HCl, 50 mM KCl, pH 8.4, triton X-100), 2 units of Taq DNA polymerase (Promega) and 0.2 mM dNTP's. Each tube contained 100 pM of nNOS upper and lower primer and the reaction mixture was covered with 100 μl of mineral oil. The thermal cycler was allowed to complete 8 cycles consisting of heat denaturation at 94 °C for 1 minute, primer annealing at 58 °C for 1 minute and polymerization at 72 °C for 1 minute. After 8 cycles the primers for cyclophilin (100 pM concentration of upper and lower) were added and the thermal cycler allowed to complete 24 additional cycles at 94 °C for 1 min, 58 °C for 1 min, and 72 °C for 1 min. A 10 μl aliquot of each RT-PCR product was subjected to electrophoresis using a 2.0% agarose gel in Trisacetate/EDTA (TAE) buffer for 50 min at 75 volts followed by staining with ethidium bromide (0.5 pg/ml). The intensities of the ethidium bromide fluorescence were determined using an IS-1000 digital imaging system (Alpha Immotech, San Leandro, CA). The area under the curve for each peak was normalized with the area of the cyclophilin peak and the values expressed as arbitrary units (AU).

Statistical Analysis: All results are expressed as means ± the standard error of the mean of the ratio of intracavernosal pressure (CCP) to the mean arterial pressure (MAP) measured at the same time. Results were analyzed using one and two way analysis of variance (ANOVA) with and without repeated measures. Following ANOVA, means were compared by Newman-Keuls post hoc analysis. Data analysis for the RT-PCR experiment was compared by Student's t test. Statistical significance was set at $P < 0.05$.

RESULTS

Testosterone treated castrate animals had blood levels of androgen of 1090 ± 105 pg/ml, while in castrated animals implanted with a single cholesterol pellet, androgen levels were 21 ± 3 pg/ml.

The results in Figure 1 show that in the rats used in these studies, as the intensity of ganglionic stimulation is increased from 1 to 6 volts, there is a step wise increase in the magnitude of the erectile response (CCP/MAP). As previously reported, the maximum response was measured at 5 or 6 volts, and the magnitude of the response in TESTO animals is greater than is measured in CASTRATE animals at equal voltage.

The experiment depicted in Figure 2 was designed to determine if the decreased magnitude of the erectile response in CASTRATE animals was due to a reduction in the availability of the amino acid, L-arginine, the substrate used by nNOS in the generation of NO. When high concentrations of this amino acid were introduced into the cavernous sinuses by injection followed by 15 minutes of infusion into the aorta, there was no increase in the CCP of the CASTRATE rats during ganglionic stimulation. This finding suggests that substrate availability is not a cause of the reduced response in the CASTRATE animals.

It has been previously reported that the intra jugular infusion of 50 μg/kg/min of L-NNA (a competitive inhibitor of NOS activity) leads to a significant decline in the magnitude of the erectile response after 45 minutes (Mills et al, 1992). In those prior studies, the inhibitor also significantly increased mean arterial blood pressure in both CASTRATE and TESTO rats. The experiments shown in Figure 3 demonstrate that a single intracavernosal injection of 200 μg

Figure 1. The erectile response, ratio of intracavernosal pressure to the mean arterial pressure (CCP/MAP), in rats that have been castrated (CASTRATE) or castrated with testosterone replacement (TESTO). The response in each rat was generated by electrical stimulation of the major pelvic ganglion using increasing voltage. Each point is the mean $\pm$ 1 SEM of measurements made in 10 rats. Asterisks indicate values significantly different from TESTO at the same voltage ($p < 0.05$).
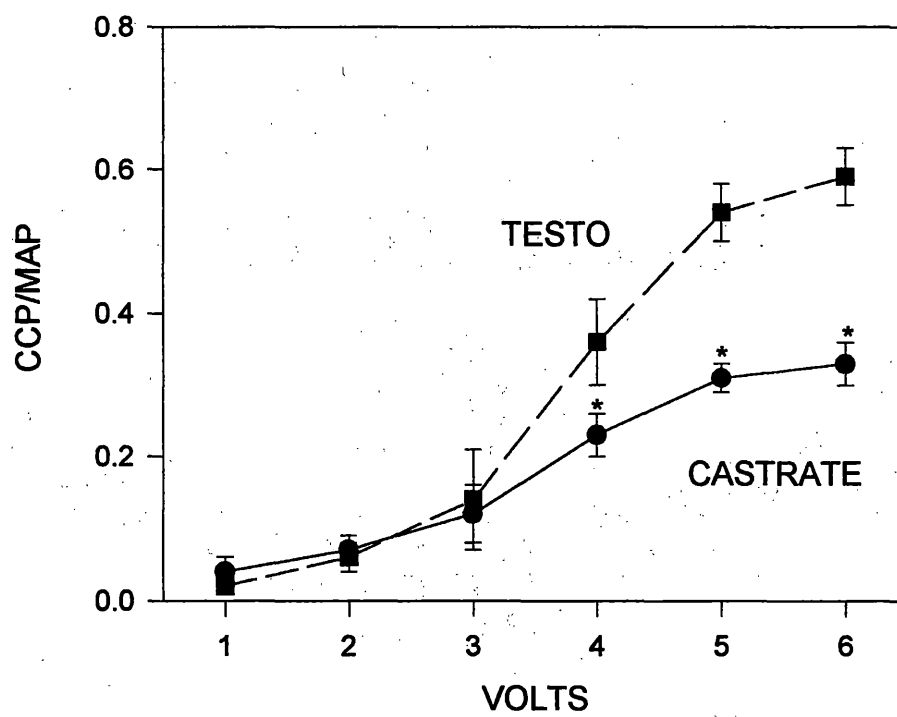
Figure 2. The erectile response in CASTRATE rats treated with intracavernosal

L-arginine by injection (INJ-25 µg/kg in 10 µl saline) followed by intra-aortic infusion at

50 µg/kg/min for 5, 10, and 15 minutes. CONT indicates the magnitude of the

intracavernosal pressure response in the same animals prior to L-arginine treatment. Each

bar represents the mean ± 1 standard error of the mean (SEM) of 4 rats. Means are not

significantly different from one another.
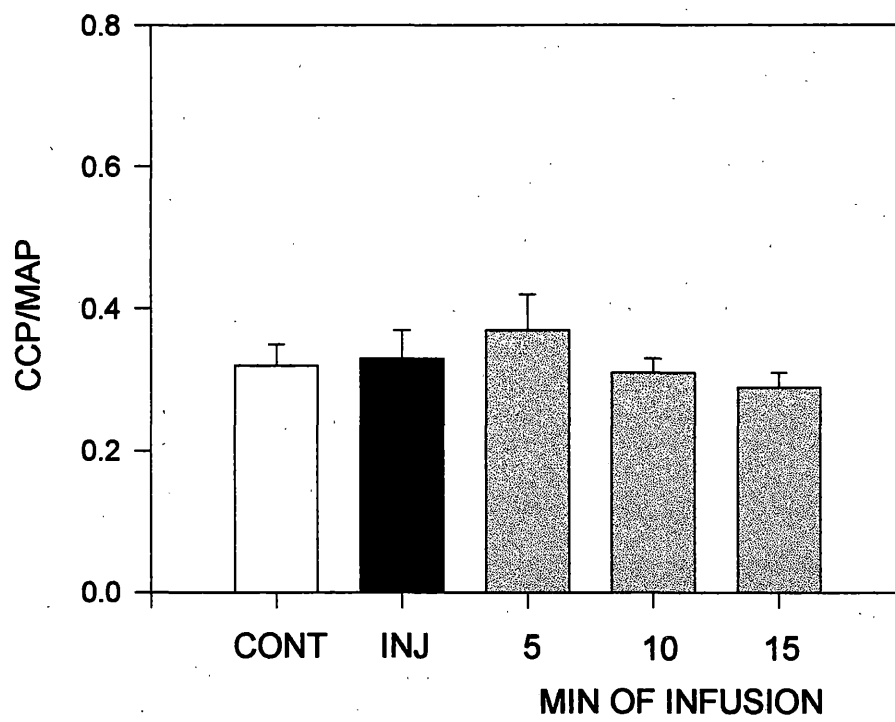
L-NNA/kg resulted in a marked decline in the intracavernosal pressure response in both TESTO and CASTRATE animals. This dose of the inhibitor did not, however, raise blood pressure (not shown). Figure 3 also shows that the marked difference in the magnitude of the stimulated erectile response between TESTO and CASTRATE animals is no longer apparent after L-NNA injection; both are sharply suppressed although intracavernosal pressure remains slightly elevated. To confirm that L-NNA is a competitive inhibitor of NOS, administration of a 100 fold excess of L-arginine partially reversed the effect of L-NNA and increased cavernosal pressures in both CASTRATE and TESTO animals. The recovery was about 80% in both TESTO and CASTRATE animals.

In the present studies, we are working under the hypothesis that androgens act primarily to regulate the quantity of NO available during erection. It follows from this hypothesis that in castrated rats with little or no androgen secretion, there would be a deficiency of NO and that exogenous NO should enhance the response in CASTRATE more than in the TESTO rats. To test this hypothesis, CASTRATE and TESTO rats were treated with intracavernous injection of sodium nitroprusside (SNP), a drug which releases NO, and measurements were made both before and after ganglionic stimulation. Figure 4 shows representative tracings of the CCP and MAP responses to ganglionic stimulation only (STIM), to SNP injection only (SNP), and to a combination of ganglionic stimulation and SNP (STIM+SNP). This experiment was repeated and the results are shown in Figure 5. During ganglionic stimulation only (STIM), the expected difference in the response in CASTRATE and TESTO rats is apparent. Following SNP injection into the

Figure 3. The electrically stimulated erectile response, ratio of intracavernosal pressure to mean arterial pressure (CCP/MAP) in TESTO and CASTRATE rats before, 10 minutes after an intracavernosal injection of 200 µg L-NNA/kg body weight and after injection of a 100 fold excess of L-arginine. Each bar represents the mean ± 1 standard error of the mean (SEM) of 5 rats. Means with different superscripts are significantly different from one another ($p < 0.05$).
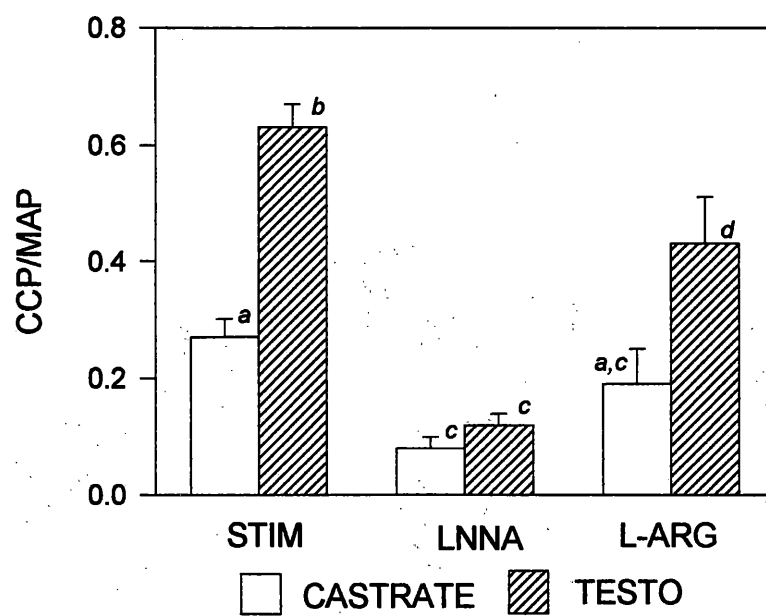
Figure 4. The effect of ganglionic stimulation and SNP on the intracavernosal pressure

response (CCP). Figure 4 shows typical erectile responses in TESTO and CASTRATE

animals to ganglionic stimulation alone, to SNP alone, and to SNP in combination with

stimulation. Major tick marks represent 1 min duration. Note that the combination of

stimulation and SNP leads to an enhanced CCP in the CASTRATE animals but not in the

TESTO rats. This experiment was repeated 6 times and the results are depicted in Figure

5.

TESTO
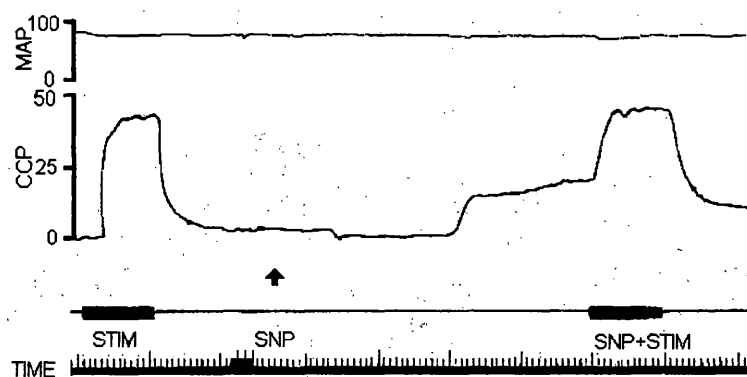
CASTRATE
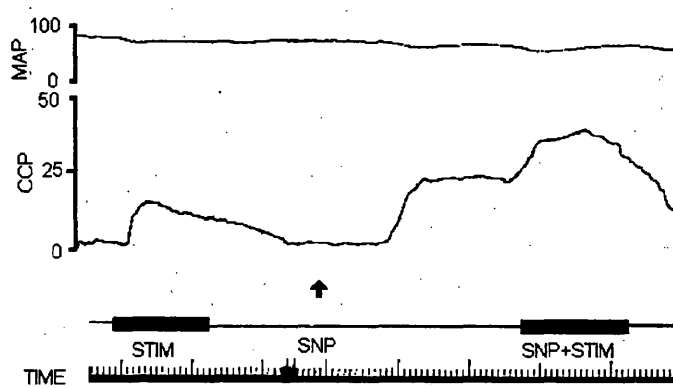
cavernous sinuses, there is a similar rise in the CCP/MAP ratio in both treatment groups. While stimulation following SNP administration (STIM+SNP) fails to increase significantly the response in TESTO rats, in the CASTRATE group the supplemental NO yields an additive effect with the magnitude of the response significantly greater than in either STIM or SNP.

We next sought to determine if androgens affect the NO production by a regulation of nNOS mRNA levels in the penile tissue. In this experiment we measured the availability of nNOS mRNA using the reverse transcription-polymerase chain reaction method (RT-PCR). Figure 6a shows the electrophoretic analysis of the RT-PCR products in TESTO and CASTRATE animals. The expected size for the PCR products for both nNOS (428 bp) and the reporter gene cyclophilin (216 bp) were obtained. The results are further analyzed in Figure 6b by computation of the ratio of nNOS peak area to cyclophilin peak area for each sample; results show that in TESTO animals the amount of nNOS mRNA in cavernosal tissue is significantly greater than the amount in CASTRATE animals.

Figure 5. The erectile response, ratio of intracavernosal pressure to intracavernosal pressure (CCP/MAP) in TESTO and CASTRATE rats resulting from electrical stimulation (STIM), intracavernosal injection of 8 μg/Kg body weight sodium nitroprusside (SNP), or stimulation following SNP injection (STIM+SNP). Each bar is the mean $\pm$ 1 standard error of the mean (SEM) of observations in 10 rats. Means with different superscripts are significantly different from one another ($p < 0.05$).
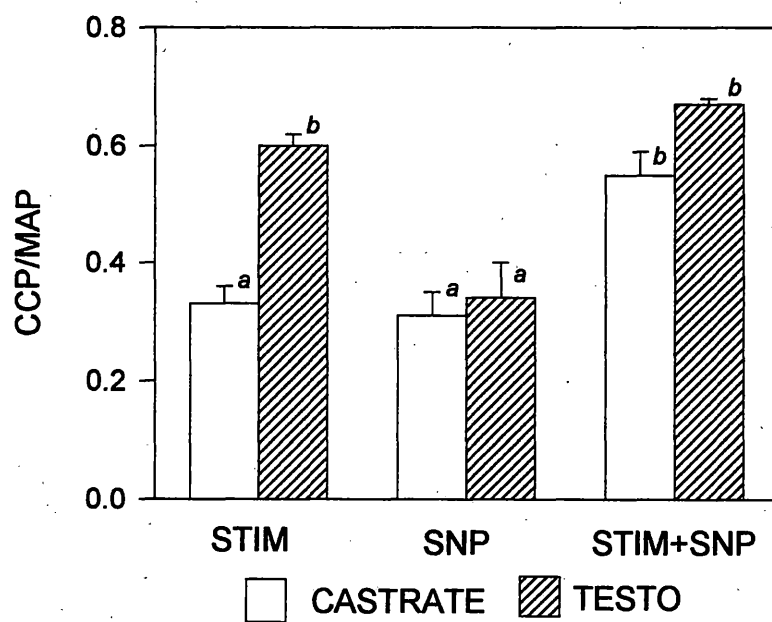
Figure 6a. Reverse transcription polymerase chain reaction (RT-PCR) amplification of cyclophilin (216bp) and the neuronal form of the nitric oxide synthase gene (nNOS) (428bp) cDNA in TESTO (above) and CASTRATE (below) animals. Lane 1-- *X174/Hae III* DNA molecular weight standards. Lanes 2-5-- co-amplified cDNA from cavernosal tissue RNA of 4 TESTO and 4 CASTRATE rats. Lane 6 -- amplified nNOS cDNA obtained from rat brain mRNA. Peak areas of the nNOS bands were normalized to the areas of the cyclophilin bands and expressed in **Figure 6b** as arbitrary units (AU) for CASTRATE and TESTO animals. Each bar is the mean ± 1 standard error of the mean (SEM) of observations in 6 rats; asterisk indicates a significantly greater AU in cavernosal tissue from TESTO than from CASTRATE rats ($p < 0.05$).

Fig. 6a
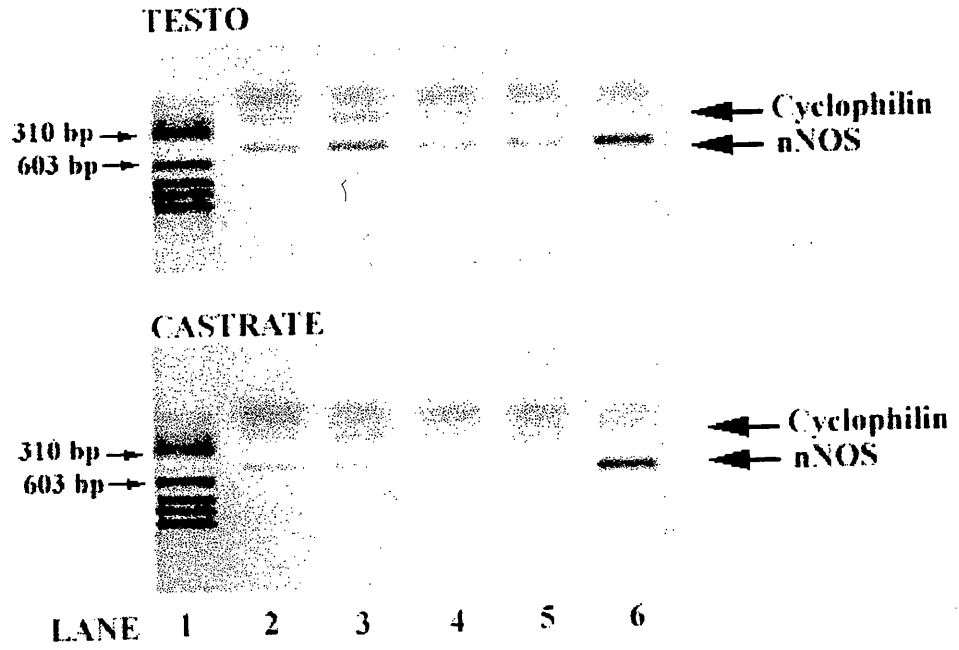
Fig. 6b
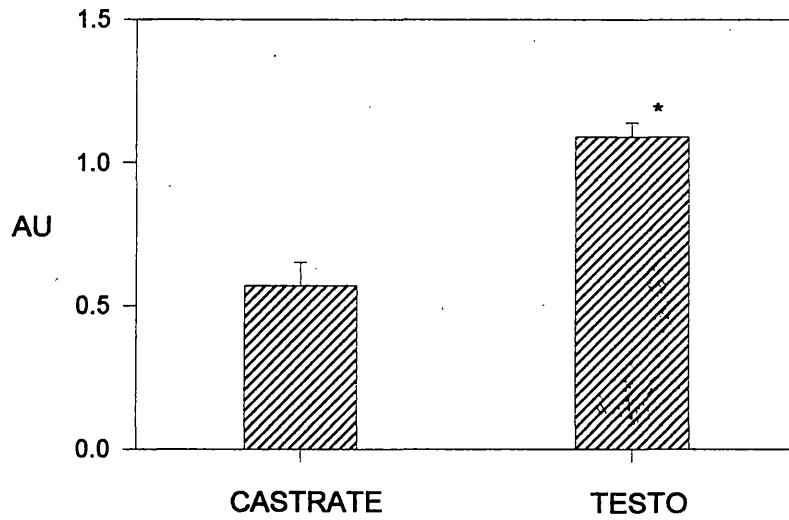
DISCUSSION

The aims of the present report are two fold; to confirm that NO is the principal mediator of the erectile response and secondly, to investigate two potential mechanisms by which androgens could regulate NO synthesis during erection. Many investigators have presented convincing evidence that NO is critical to erection, and while our studies use a somewhat dissimilar approach, the results serve to confirm the general importance of NO in erection. The mechanistic studies, on the other hand, extend our understanding of how androgens act by showing that substrate availability is not regulated by androgens while expression of the nNOS gene appears to be under direct androgenic control.

As reports from this and other laboratories have shown, the magnitude of the intracavernosal pressure rise during erection increases as the voltage applied to the autonomic innervation of the cavernosal vasculature is increased. Furthermore, the pressure rise in castrated animals falls well below the increase in intact rats or animals treated with exogenous androgen. These studies have led to the conclusion that there is an androgen dependent and androgen independent portion of the erectile response in rats. The experiments using L-NNA show that both the androgen dependent and the androgen independent portions are mediated by NO since both are diminished by more than 80% with only 10 minutes of treatment with the inhibitor. In prior studies from this laboratory, systemic infusion (jugular vein) of L-NNA (50 µg/kg/min for 45 min) led to a significant reduction in the erectile response but at this rate of drug delivery, systemic blood pressure was significantly increased. In the present study, mean arterial pressure was not affected by the intracavernosal delivery of L-NNA even though there was a marked reduction in the CCP. Furthermore, the inhibitor reduced the CCP to the same extent in both

CASTRATE and TESTO rats indicating that both portions of the response (androgen dependent and androgen independent) are mediated by NO. However, the small increase in CCP that remained after L-NNA treatment indicates that the dose of L-NNA was sub-optimal or that a small portion of the erectile response involves a pathway independent of NO.

The experiment depicted in Figure 2 was designed to determine if the decreased magnitude of the erectile response in CASTRATE animals was due to a reduction in the availability of the amino acid, L-arginine, the substrate used by nNOS in the generation of NO. When high concentrations of this amino acid were introduced into the cavernous sinuses by injection or by 15 minute infusion into the aorta, there was no increase in the CCP of the CASTRATE rats during ganglionic stimulation. Although circulating levels of L-arginine were not measured, this finding suggests that substrate availability is not a cause of the reduced response in the CASTRATE animals and that testosterone is not acting to increase substrate availability to the NOS reaction.

SNP acts as a NO donor (Martinez-Pineiro et al, 1993) and the sustained action of this drug or the relatively low rate of clearance from the corpus cavernosum results in a prolonged increase in intracavernosal pressure. Injection of SNP into the cavernosum without ganglionic stimulation resulted in increases in intracavernosal pressure which were similar in both TESTO and CASTRATE animals. That the magnitude of the response to SNP was nearly identical in the two treatment groups suggests that there is no difference in the responsiveness of the cavernosal mechanisms to NO. Rather, it suggests that the difference in the erectile response to ganglionic stimulation in TESTO and CASTRATE

rats may depend solely on differences in the availability of NO, due possibly to differences in the capacity to synthesize NO. When the levels of NO were elevated by the administration of SNP and the ganglion stimulated, TESTO treated animals exhibited no significant increase in cavernosal pressure over that seen with stimulation alone whereas in CASTRATE animals the pressure increased to that observed in TESTO rats. This finding can be interpreted to mean that the erectile response can be augmented with additional NO in the CASTRATE animals where NO is deficient but not in TESTO rats where NO levels are not limited. Some CASTRATE animals received an additional intracavernosal injection of 16 μg SNP/kg body weight of SNP but the additional NO failed to further increase the response suggesting that the dose of SNP used was optimal (data not shown).

The final series of experiments was performed to determine if androgens alter the availability of NO by regulation of nNOS mRNA levels. In this experiment expression of the nNOS gene in cavernosal tissue was measured using the reverse transcription polymerase chain reaction method. Our studies showed that in cavernosal RNA preparations, there was significantly less nNOS mRNA in CASTRATE animals than in TESTO animals. This finding extends the work of Chamness et al, (1995) who reported that nNOS protein levels in the rat penis decrease 57% following castration and with the studies of Zvara et al, (1995) who have demonstrated that castration decreases by 60% NADPH diaphorase staining in the NANC nerve fibers innervating the corpus cavernosum. The fact that castration does not lead to a total disappearance of nNOS mRNA may serve to explain the androgen dependent and androgen independent portions

of the erectile response.   From these results we propose that in CASTRATE animals, a basal level of nitric oxide is released by nerve fibers in response to ganglionic stimulation which results in vasodilation and a partial erectile response. Androgens increase the amount of nNOS mRNA, by enhancing nNOS gene expression or by decreasing mRNA degradation, resulting in a greater amount of enzyme available for the production of NO in the rat penis.

CHAPTER 3

Specific Aim 2: Determine if androgens facilitate penile erection by activating a NO

independent pathway.

PREFACE

Exploration of Other Possible Vasodilators Pathways in the Rat Penis: Although NO

is considered to be the predominant vasodilator in the penis, questions remain as to the

possibility of other vasodilatory pathways acting independently of NO in the erectile

response. Using specific stimulators and inhibitors, cAMP dependent pathways resulting

in erection have been demonstrated in primates and humans, but their importance has not

been established in rats. Prostaglandin E_1 (PGE_1), considered a cAMP pathway activator,

results in full tumescence in humans (Knispel et al, 1994; Shenfeld et al, 1995), causes

only mild tumescence in dogs (Aboseif et al, 1989), but has no effect on rabbits (Knispel et

al, 1991) or rats (Martinez-Pineiro et al, 1993) suggesting the cyclic AMP pathway is not

involved in the erectile response in rodents. However, in rats, it is intriguing that

papaverine (a phosphodiesterase inhibitor) produces erection in the presence of methylene

blue, cyclic GMP inhibitor (Martinez-Pineiro et al, 1993). The fact that papaverine, via

inhibition of phosphodiesterase activity, results in tumescence when injected into the

corpus cavernosum of rats indicates pathways which are not mediated by NO can be

stimulated to produce tumescence.

The following is the text of a manuscript entitled "Androgenic Maintenance of the Rat Erectile Response Via a Non Nitric Oxide Dependent Pathway," which has been accepted for publication by the *Journal of Andrology*. The authors of this manuscript are Christopher M. Reilly, Ronald W. Lewis, Vivienne S. Stopper and Thomas M. Mills.

ABSTRACT

Prior studies have demonstrated that the erectile response in the rat penis is androgen

dependent and is mediated by nitric oxide (NO), the neurotransmitter synthesized by the

enzyme nitric oxide synthase (NOS). The present studies used L-nitro-L-arginine methyl

ester (L-NAME), an inhibitor of NOS, to determine if androgens also regulate alternative

pathways leading to the erectile response but not mediated by NO. Castrated rats which

were treated with L-NAME (L-NAME CASTRATE) exhibited little or no increase in

intracavernosal pressure in response to stimulation of the major pelvic ganglion. This

ganglion controls blood flow into the penis and when stimulated normally leads to

erection. However, when castrated animals were treated with testosterone along with

L-NAME (L-NAME TESTO), the animals responded to the ganglionic stimulation with

increased intracavernosal pressure. This finding suggests that there are other androgen

dependent pathways which lead to penile erection but are not mediated by NO. Erection

occurred in both L-NAME CASTRATE and L-NAME TESTO rats in response to

intracavernosal injection of sodium nitroprusside (a NO donor drug) proving that the NO

responsive mechanisms were unaffected by the inhibition of NOS activity. To investigate

further the nature of this NO independent pathway, L-NAME CASTRATE and L-NAME

TESTO rats were treated with either zaprinast (a specific phosphodiesterase 5 inhibitor)

which would block the breakdown of cGMP to 5'GMP or rats were treated with

methylene blue (an inhibitor of guanylate cyclase) to prevent the synthesis of cGMP.

Zaprinast treatment led to increased erectile response in L-NAME TESTO rats, but not in

L-NAME CASTRATE rats, demonstrating that androgen sensitive alternative pathways

increased guanylate cyclase activity. Methylene blue inhibited the erectile response in all treatment groups showing that cyclic GMP is critical to the NO independent pathway as well as the NO dependent pathway. Taken together, these results support the hypothesis that androgens maintain the erectile response by alternate pathways including one which is independent of NO but involves the synthesis of cyclic GMP.

Key words: Penile erection, testosterone, cGMP, phosphodiesterase 5.

INTRODUCTION

It is generally accepted that NO, a neurotransmitter which originates in nonadrenergic noncholinergic (NANC) nerve fibers of the penis, is the principal vasodilator during the erectile response. Several authors have demonstrated that inhibitors of NO abolish erectile function (Reilly et al, 1997; Mills et al, 1992; Rajfer et al, 1992; Ignarro et al, 1990; Holmquist et al, 1991; Pickard et al, 1991) or have shown tumescence in response to NO releasing drugs (Holmquist et al, 1993; Kim et al, 1991; Ignarro et al, 1990; Burnett et al, 1995; Burnett et al, 1992). Several authors have reported that androgens maintain erectile function by regulating levels of NO (see review by Mills et al, 1996a). Recently, we have reported that testosterone maintains the quantity of neuronal nitric oxide synthase (nNOS) mRNA in the rat penis (Reilly et al, 1997).

Although NO is considered to be the predominant vasodilator in the penis, there are also other vasodilatory pathways in the erectile response. Prostaglandin E_1 (Hanawa 1991), vasoactive intestinal peptide (Wang et al, 1993; Gozes and Fridkin 1992), neuropeptide Y (Kawano et al, 1993; Carrillo et al, 1991), calcitonin gene related peptide (Alaranta et al, 1991; Stief et al, 1990), and endothelin-1 (Holmquist et al, 1992; Garcia-Pascual et al, 1993; Persson et al, 1992) have been proposed as possible vasoactive neurotransmitters during erection. Although administration of prostaglandin E_1 results in full tumescence in humans, via a cAMP second messenger pathway, (Knispel et al, 1994; Shenfeld et al, 1995), no enhancement of the erectile response has been demonstrated in rats (Martinez-Pineiro et al, 1993) indicating that cAMP dependent pathways may not be normally involved. However, in rats, papaverine (a non specific phosphodiesterase

inhibitor) produces erection in the presence of methylene blue, an inhibitor of guanylate cyclase activity (Martinez-Pineiro et al, 1993). This finding shows that if the cGMP pathway is inhibited, cAMP pathways can be stimulated to produce tumescence. Taken together these studies suggest the possibility that alternate pathways are present that become evident only when the predominant nNOS pathway is inhibited. Such alternative pathways may involve other NOS isoforms including endothelial NOS (eNOS), or ineducable NOS (iNOS) or a non NO pathway. The presence of alternative pathways could explain why transgenic mice without nNOS gene expression (nNOS knock out mice) exhibit normal erections (Huang et al, 1993; Nelson et al, 1995). The present studies were designed to test the hypothesis that in the absence of NO synthesis, testosterone supports an alternative, cGMP dependent pathway leading to erection.

MATERIALS AND METHODS

Animals: Male Holtzman rats (Harlan), retired breeders weighing 400-600 gms were used in these studies. Testosterone pellets were made in the laboratory using a pellet press. Each animal was surgically castrated under ether anesthesia and implanted with either a 3 mm (approx 5 mg) pellet composed of 50% testosterone and 50% cholesterol (group designation SM TESTO), a 5 mm (approx 15 mg) pellet composed of 50% testosterone and 50 % cholesterol (group designation LG TESTO), or a control pellet of 100 % cholesterol (group designation CHOL). Additionally, some animals were given L-nitro-L-arginine methyl ester (L-NAME) in their drinking water for one week at a concentration of 0.67 g/L starting on the day of castration. This concentration and duration of L-NAME treatment has been previously determined to abolish NO production by inhibiting NOS activity (Pollock et al, 1993). Experimental procedures were performed during the L-NAME treatment 6 - 9 days after pellet implantation. At the end of each experiment, blood was collected from the carotid artery for the measurement of circulating levels of testosterone by radioimmumoassay as previously described (Melner and Abney 1980). Animals were maintained in an AAALAC accredited laboratory facility with animals use protocols and justifications approved by the Committee for Animal Use in Research and Education at the Medical College of Georgia.

Preparation of Animals for Measurement of Intracavernosal Pressure: The method for stimulation and measurement of the erectile response in this laboratory has been previously described (Mills et al, 1992; Mills et al, 1994; Mills et al, 1996). Briefly, the rat was

anesthetized with an intramuscular injection of ketamine (87 mg/Kg) combined with

xylazine (13 mg/kg) and maintained on supplemental injections of ketamine and xylazine

as needed. The carotid artery was cannulated with a blunted 18 gauge needle with PE 190

tubing attached to a pressure transducer and connected to a multichannel recorder to

continuously record mean arterial blood pressure (MAP). The abdominal cavity was

opened, the viscera retracted, covered in saline soaked sponges and wrapped in cellophane

to reduce evaporation and to help maintain body temperature. The right dorsal prostate

was cleared from fat and adhering fascia to expose the right cavernosal nerve and the

major pelvic ganglion. The shaft of the penis was cleared of the overlying fascia and the

left corpus cavernosum was cannulated by insertion of a saline filled 30 gauge needle

connected to PE 10 tubing for intracavernosal drug delivery, Next, the right corpus

cavernosum was cannulated by insertion of a 30 gauge needle attached via PE 10 tubing

to a pressure transducer and connected to a multichannel recorder to continuously record

intracavernosal pressure (CCP).

Measurement of the Erectile Response: Stainless steel bipolar electrodes were positioned

on the pelvic nerve and the ganglion using a micro manipulator and the electrodes were

connected to a Grass stimulator. When possible, proper electrode placement was

established by determining the position of electrodes that produced the maximal CCP

with minimal alteration of mean arterial pressure (MAP) at a 5 volt stimulation (pulse

duration -5 msec, frequency -12 Hz.). After electrode placement, each animal was

subjected to stimulatory voltages from 1 to 6 volts for one minute with a recovery time of

1 minute after each stimulation. Previous studies from this laboratory have determined the maximal erectile response occurs at a stimulatory voltage of 5 or 6 volts (Mills et al, 1992). Simultaneous recordings were made of MAP, CCP and time.

Intracavernosal Drug Administration: The following drugs were injected singly into the left corpus cavernosum: L-nitro-L-arginine (L-NNA), 200 mg/kg body weight (BW) was injected in 5 µl saline 10 minutes prior to ganglionic stimulation. Sodium nitroprusside (SNP), 8 µ g/kg BW, was injected in 1 µl of saline 3 minutes prior to ganglionic stimulation. Zaprinast, 0.1 mg/kg BW, was injected in 25 µl in an alkaline saline solution (pH 10.5) 3 minutes prior to ganglionic stimulation. Methylene blue, 300 µg/kg BW, was injected in 30 µl saline five minutes before ganglionic stimulation. For control in the zaprinast experiment the same volume of alkaline saline was injected. In all other drug injection experiments, an equal volume of saline was injected as control at the appropriate time before ganglionic stimulation. In order to minimize the effects of multiple drug exposure, each animal was injected with no more than two drugs during the experiment. Furthermore, prior to administration of the second drug, the animal was allowed to recover from the first drug. All experimental procedures were completed within 2 hours of the initial anesthesia.

Statistical Analysis: Results in the methylene blue and the zaprinast experiments were compared by paired variable students t-test. All other results were analyzed by one way,

or 2 way analysis of variance (ANOVA), followed by Newman-Keuls post hoc analysis. Results are expressed as the mean $\pm$ one standard error of the mean. Statistical significance was set at $P < 0.05$.

RESULTS

The dose of L-NAME used in these studies was based on published reports by Pollack et al, (1993) who reported that this concentration of the drug given in the drinking water of rats resulted in marked hypertension within a one week period. Preliminary studies revealed that L-NAME treatment led to highly variable blood levels of androgen in non-castrated rats. Accordingly, in order to maintain stable blood androgen levels, animals were castrated and implanted with a single large or small testosterone pellet. Control animals received only a single cholesterol pellet after castration.

The androgen levels in rats one week after castration and implantation of a single cholesterol pellet - 50 $\pm$ 10 pg/ml (CASTRATE), after implantation of a small testosterone pellet - 1050 $\pm$ 100 pg/ml (SM TESTO), and following implantation of a large testosterone pellet - 6000 $\pm$ 350 pg/ml (LG TESTO). L-NAME administration did not alter testosterone levels in any of the treatment groups.

In several instances during these experiments, individual rats experienced a spontaneous erection that occurred without ganglionic stimulation. Table 1 shows the number of rats exhibiting a spontaneous erection in each of the treatment groups. This table shows that there was no significant difference in the number of animals showing spontaneous erections in the TESTO, CASTRATE and L-NAME -TESTO groups whereas the L-NAME-CASTRATE animals had significantly fewer spontaneous erections with only a single event in one animal. Comparisons of the magnitude of the erectile response (CCP/MAP) during spontaneous erections and during erections induced by ganglionic stimulation are shown in Figure 1. There is no significant difference between

Table 1. The number of rats exhibiting a spontaneous erection compared to the total

number of rats (SPONT/TOTAL) in TESTO, CASTRATE, L-NAME-TESTO and

L-NAME-CASTRATE animals. Asterisk indicates a significant difference from

TESTO rats ($P<0.05$).

TESTO	CASTRATE	L-NAME-TESTO	L-NAME-CASTRATE
7/26 (27%)	9/27 (33%)	9/30 (30%)	* 1/11 (9%)

the magnitude of the spontaneous erection and the induced erection in the TESTO, CASTRATE, and L-NAME TESTO rats. The magnitudes of both the spontaneous and induced erectile responses in the TESTO rats were significantly greater than the responses in CASTRATE and L-NAME-TESTO rats.

The animals receiving L-NAME in their drinking water had significantly higher MAP values (153 ± 7 mmHg in L-NAME-TESTO and 159 ± 8 mmHg in L-NAME-CASTRATE) than the control animals drinking untreated water (128 ± 5 mmHg in TESTO and 128 ± 4 mmHg in CASTRATE) $P<0.05$. However, there was no difference between TESTO and CASTRATE animals independent of L-NAME treatment.

The experimental results depicted in Figure 2 show the erectile response to ganglionic stimulation in CASTRATE and TESTO rats with and without L-NAME treatment. This figure shows that although the CASTRATE rats show a partial erectile response to ganglionic stimulation, the magnitude of the response is nearly doubled when the castrated animals are given androgen (SM TESTO). Furthermore, additional androgen (LG TESTO) did not further enhance erectile function (data not shown). This figure also shows that the erectile response in L-NAME-CASTRATE rats is virtually absent but when testosterone is given (L-NAME-TESTO), the response partially returns. In this instance when the L-NAME treated rats were given additional androgen (SM TESTO or LG TESTO) the response to ganglionic stimulation was enhanced further. While the implantation of a large testosterone pellet increased the blood androgens to super physiologic levels, it can be argued that elevated steroid hormone levels may be required to exaggerate a subtle hormonal effect. Accordingly, in all subsequent studies

Figure 1. Comparisons of the magnitude of spontaneous erections to erections resulting

from ganglionic stimulation in TESTO and CASTRATE rats with and without L-NAME

treatment. Each bar represents the mean $\pm$ 1 SEM of measurements in at least 7 rats.

Means with different letters are significantly different from each other. *Since only a

single L-NAME CASTRATE animal elicited an erectile response the animal was not

computed in the statistical analysis.
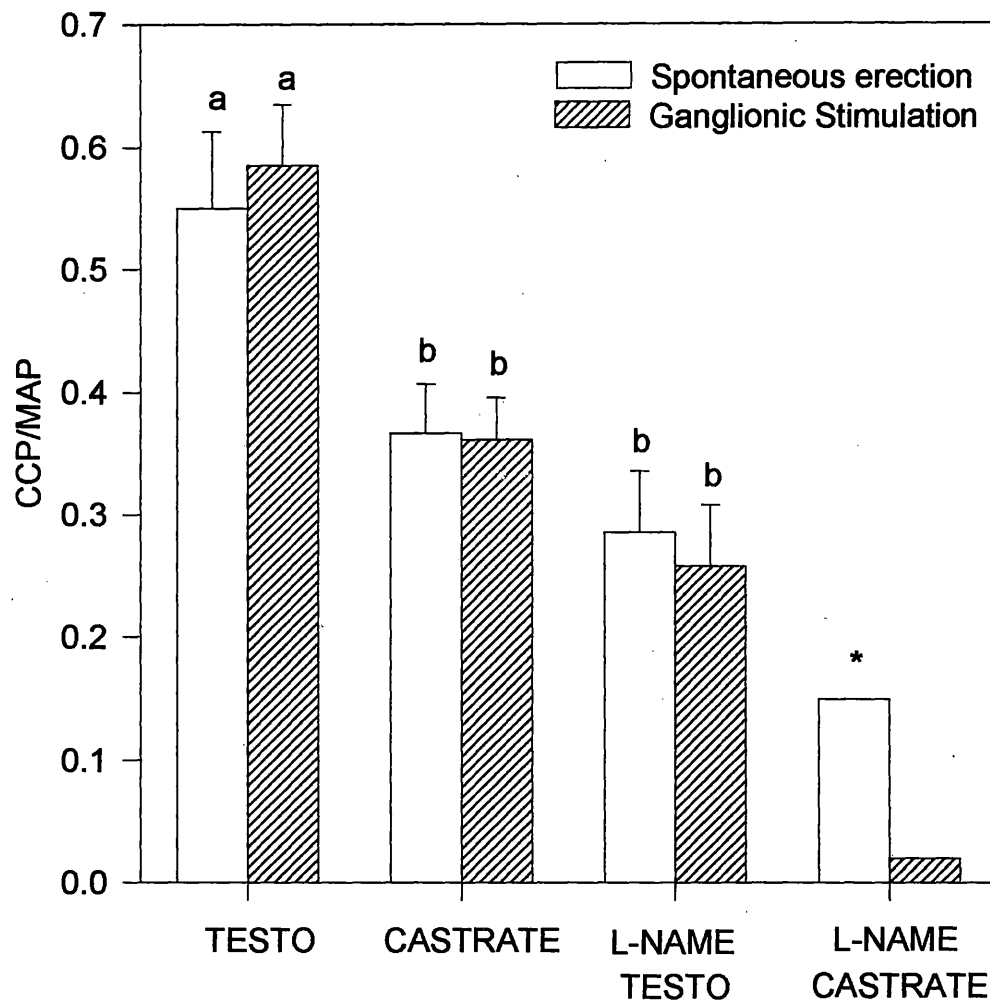
Figure 2a. The erectile response (CCP/MAP) to increased levels of ganglionic stimulation in castrated rats (CASTRATE) and castrated rats receiving a 3 mg testosterone replacement (TESTO). Each point is the mean $\pm$ 1 SEM of measurements made of at least 5 rats. Asterisks indicate values significantly different from TESTO at the same voltage (P<0.05). **Figure 2b** shows the erectile response of CASTRATE and TESTO rats that received 0.67 g/l L-NAME in the drinking water over the same duration of time. In this instance the (CCP/MAP) ratio was increased with additional androgen (SM TESTO vs LG TESTO). Each point is the mean $\pm$ 1 SEM of measurements made in at least 10 rats. Asterisks indicate values significantly different from LG TESTO at the same voltage (P<0.05).

Fig. 3a (untreated water)

Fig. 3b (0.67g/l L-NAME in the drinking water)

- LG TESTO
- CASTRATE
- SM TESTO

VOLTS
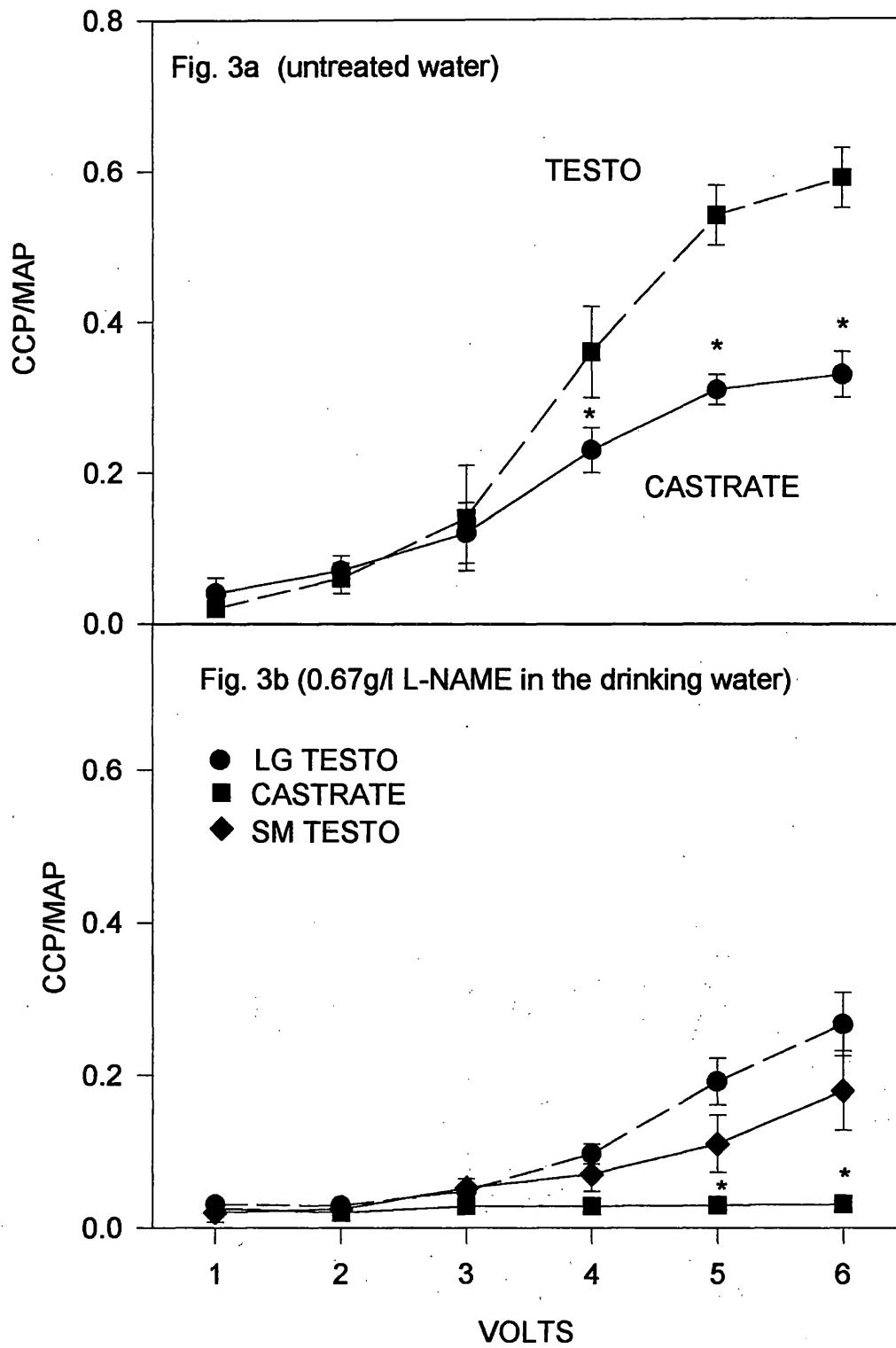
reported here, rats received a large testosterone pellet to maintain high levels of androgen in the blood.

To insure that 1 week of L-NAME treatment completely suppressed NO synthesis, L-NAME treated animals were also given another NOS antagonist. In this experiment 200 mg L-NNA /kg BW was injected directly into the corpus cavernosum of L-NAME-TESTO rats. We have previously reported that this amount of L-NNA nearly abolished the erectile response in both CASTRATE and TESTO animals (Reilly et al, 1997). Figure 3 demonstrates that when L-NAME-TESTO rats are given 200 μg L-NNA/kg BW, the magnitude of the erectile response remains unchanged confirming that NOS activity is completely suppressed by L-NAME treatment only.

We next sought to determine if one week of treatment with L-NAME altered the responsiveness to a NO donor drug. We have previously shown that 8 μg/Kg BW of SNP injection into the penis results in tumescence (Reilly et al, 1997). Figure 4 shows that this dose of SNP results in a similar rise in CCP/MAP in both the L-NAME-CASTRATE and L-NAME-TESTO animals. Furthermore, while ganglionic stimulation alone failed to increase the intracavernosal pressure in L-NAME-CASTRATE animals, administration of SNP to these animals resulted in tumescence similar to that of L-NAME-TESTO rats. This experiment proves that the tissue remains fully responsive to NO during L-NAME treatment.

To determine if testosterone acts to support erectile function by increasing cGMP levels in the absence of NO, the effect on the erectile response of a specific (Boolell et al, 1996) phosphodiesterase 5 (PDE 5) inhibitor and an inhibitor of guanylate cyclase were

Figure 3. The erectile response (CCP/MAP) following L-NNA administration in castrated rats receiving a large testosterone replacement pellet and L-NAME for a one week period. PRE-LNNA indicates the magnitude of the erectile response before intracavernosal injection of 200 ug/kg BW of the NOS inhibitor L-NNA. POST-L-NNA is the erectile response measured 5 minutes after injection. Each bar represents the mean $\pm$ 1 SEM of 6 animals. There was no significant difference.
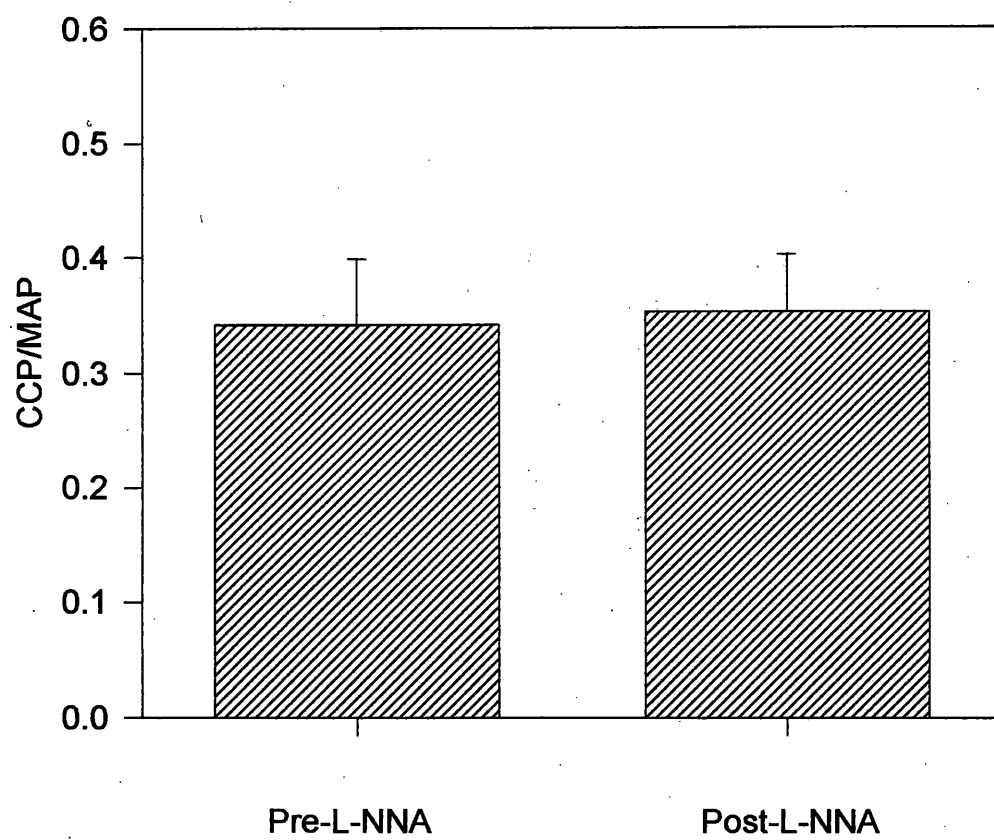
Figure 4. The erectile response (CCP/MAP) in L-NAME-TESTO and

L-NAME-CASTRATE rats resulting from ganglionic stimulation (STIM), intracavernosal

injection of 8 mg/Kg BW sodium nitroprusside (SNP), or ganglionic stimulation following

SNP injection (STIM+SNP). Each bar is the $\pm$ 1 SEM of observations of at least 6

animals. Means with different letters are significantly different from one another (p<0.05).
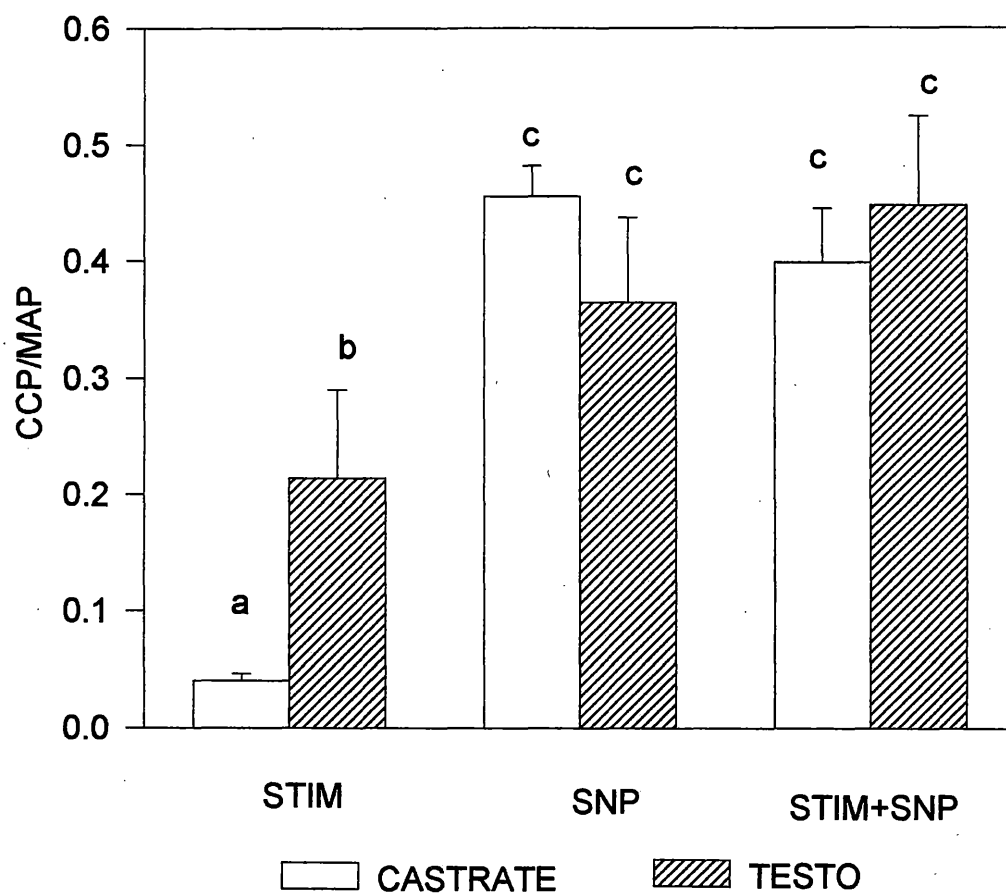
investigated in L-NAME-TESTO rats. We reasoned that since PDE 5 is the major PDE in

penile tissue, (Lugnier and Komas 1993) then the administration of a specific inhibitor

would prevent the degradation of cGMP and increase intracavernosal pressure in

L-NAME-TESTO rats during ganglionic stimulation. Figure 5a shows that when 0.1

mg/Kg BW zaprinast, was injected into the corpus cavernosum of L-NAME-TESTO

animals, there was a significant increase of CCP in L-NAME-TESTO rats. Zaprinast

administration did not, however, increase the erectile response to ganglionic stimulation in

L-NAME-CASTRATE rats (data not shown). Figure 5b shows that inhibition of

guanylate cyclase by injection of methylene blue into the corpus cavernosum resulted in a

significant decrease in intracavernosal pressure in L-NAME-TESTO rats. This

observation suggests that in rats in which NOS activity has been suppressed, the

testosterone supported erectile response is cGMP dependent.

Figure 5a. The erectile response in L-NAME-TESTO rats following electrical stimulation of the major pelvic ganglion before and after zaprinast administration (PRE-ZAP and POST-ZAP). Each line represents one animal and shows the change in CCP/MAP caused by drug administration for that animal. The values after zaprinast treatment are significantly different from those before drug injection (P<0.05). **Figure 5b.** The erectile response in L-NAME-TESTO rats following electrical stimulation of the major pelvic ganglion before and after methylene blue administration (PRE-MET and POST-MET). Each line represents one animal and shows the change in CCP/MAP caused by drug administration for that animal. The values after methylene blue treatment are significantly different from the values before drug injection (P<0.05).

Fig. 5a
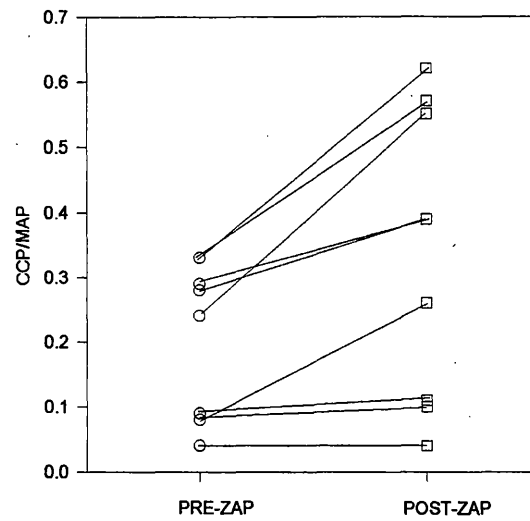
Fig. 5b
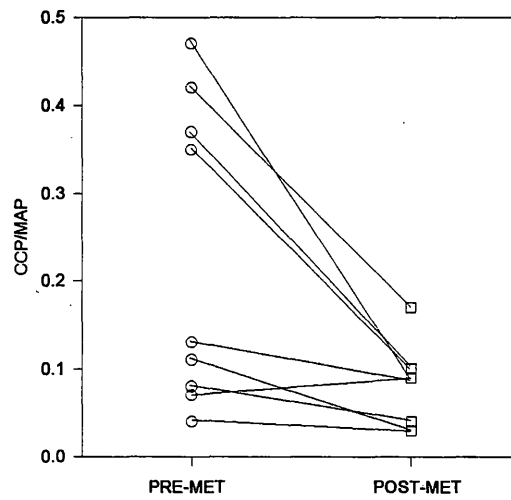
DISCUSSION

In prior studies, we have shown that castration results in a marked decrease in the erectile response, and testosterone replacement restores the response to that observed in intact animals (Mills et al, 1992). To characterize further the role of testosterone in maintaining erectile function we demonstrate that in the absence of available NO an erectile response can be induced, and that high levels of androgen are required to maintain this NO independent erectile response. L-NAME was used to block the production of NO; this drug has been shown to lead to hypertension when added to the drinking water (Pollock et al, 1993). Additionally, in cavernosal tissue, L-NAME blocks NOS activity measured by arginine conversion to NO and citrulline (Penson et al, 1996; Lugg et al, 1995). Although NO production was fully blocked in the present study, some animals maintained partial erectile function in response to stimulation of the MPG. In addition, despite the L-NAME blockage of NOS activity there were occasionally spontaneous erections in several of the animals. These spontaneous erections were marked by a rapid increase in the intracavernosal pressure lasting only a few seconds followed by a rapid decline back to baseline values. When direct comparisons were made, we found that the magnitude of the spontaneous erectile response (CCP/MAP) was the same as the response invoked by ganglionic stimulation. This observation suggests that in our experimental procedures, although the electrically induced erections occur *ex copula*, the responses are similar in magnitude to natural erections. Because of this, we feel that this model is appropriate to study factors which influence erectile function in rats.

Several authors have reported that testosterone acts to maintain the enzyme NOS and the resulting NO leads to relaxation of cavernosal and arteriolar smooth muscle (Reilly et al, 1997; Zvara et al, 1995; Chamness et al, 1995; Lugg et al, 1995). Others have even suggested that complete androgen withdrawal results in a cessation of erectile function only by decreasing NOS activity rather than decreasing the availability of NOS (Penson et al, 1996).

While L-NNA injection into the penis had previously been shown to decrease intracavernosal pressure in TESTO and CASTRATE animals (Reilly et al, 1997), in the present studies injection of this NOS inhibitor failed to diminish the erectile response further showing that the L-NAME inhibition of NOS activity was maximal. The action of testosterone to maintain partial erectile responsiveness in the NO depleted animals can therefore be interpreted to mean that testosterone is acting to maintain erectile function through an alternate, NO independent pathway.

PDE 5, a phosphodiesterase which inactivates cGMP by converting it to 5'GMP has been reported to be the predominant PDE isoenzyme in the penis (Boolell et al, 1996). Several researchers have demonstrated that the erectile response is increased following treatment with specific PDE 5 inhibitors (Trigo-Rocha et al, 1993; Rajfer et al, 1992; Trigo-Rocha et al, 1994). In our experiments, inhibition of PDE 5 likewise increased erectile responsiveness in CASTRATE and L-NAME-TESTO animals. In CASTRATE animals this could be due to endogenous NO activation of guanylate cyclase, since castration does not cause complete loss of NOS activity (Reilly et al, 1997; Chamness et al, 1995; Zvara et al, 1995). However, in the L-NAME-TESTO animals in which there is

no NO production, testosterone appeared to increase production of cGMP leading to erection by some pathway which was independent of NO. This increase in cGMP did not occur in the absence of testosterone (L-NAME-CASTRATE) since there was no increase in intracavernosal pressure following the administration of the cGMP synthesis inhibitor to these animals.

Methylene blue inhibits guanylate cyclase activity (Lugnier and Komas 1993; Kawada et al, 1994; Trigo-Rocha et al, 1993) and has been reported to diminish the erectile response in rats. In addition to inhibiting guanylate cyclase activity, methylene blue may also inhibit NOS directly (Mayer et al, 1993). We also observed a decrease in the erectile response in L-NAME-TESTO rats given methylene blue suggesting that testosterone supports a pathway which includes the activation of guanylate cyclase. Although the intracavernosal pressure decrease following methylene blue injection was significant, the penile tissue retained partial responsiveness to ganglionic stimulation suggesting that there may be other testosterone dependent pathways that do not involve the guanylate cyclase/cGMP second messenger system. Additionally, the role of the adenylate cyclase/cAMP second messenger pathway remains to be fully explored since papaverine results in full tumescence in rats via a cAMP dependent system (Martinez-Pineiro et al, 1993).

In summary these studies show that in rats in which NOS activity has been completely suppressed, testosterone maintains an erectile response. It is likely that the androgen activates pathways that involve the enzyme guanylate cyclase but other secondary or minor pathways may also depend on the availability of testosterone.

CHAPTER 4

Specific Aim 3: Determine if testosterone enhances the erectile response by decreasing the cavernosal sensitivity to α adrenergic stimulation.

PREFACE

Effects of Androgens on the Responsiveness of Vascular Smooth Muscle: In addition to the actions of vasodilators, a decrease in sympathetic tone is required to permit increased blood flow into the cavernous sinuses leading to erection (Phoenix and Chambers 1986). Sympathetic tone in the penis is the result of norepinephrine release from adrenergic nerve fibers that innervate cavernosal tissue. Norepinephrine binds to the α_1 receptors initiating a cascade of events resulting in smooth muscle contraction. This process involves the α_1 adrenergic receptor associating with a Gq protein. The Gq protein is comprised of an α, β, and γ subunit. When the norepinephrine activated receptor associates with its G protein it releases GDP allowing a GTP to bind the α subunit. The activated Gqα-GTP complex then dissociates from the β and γ subunits and binds to a membrane bound enzyme (phospholipase C) that catalyses the conversion of phosphatidyl inositol to inositol 1, 4, 5, triphosphate (IP_3) and diacyl glycerol (DAG). IP_3 then releases Ca^{2+} from the sarcoplasmic reticulum, intracellular storage vacuoles, and opens ion gated Ca^{2+} channels to increase the intracellular levels of Ca^{2+}. The Ca^{2+} then complexes with calmodulin to activate myosin light chain kinase allowing ATP to bind the myosin light chain resulting in contraction of the smooth muscle cell (Barada and McKimmy 1994).

Numerous reports confirm that norepinephrine is the detumescent factor that causes contraction of the vascular smooth muscle in the arterioles and trabeculae of the corpus cavernosum and point to the necessity for a decrease in sympathetic tone for erection to occur (Giuliano et al, 1993a; Susset et al, 1989; Kirkeby et al, 1989; Diederichs and Lue 1991; Blum et al, 1985; Holmquist et al, 1990; Christ et al, 1992; 55; Adaikan and Ratnam. 1988; Truss et al, 1994; Levin et al, 1994).

Androgens have been demonstrated to influence smooth muscle cells in various ways. Zhang and coworkers (1991), suggested steroid hormones regulate anion metabolism and Ca^{2+} transport across smooth muscle cell membranes. Others have demonstrated an androgenic regulation on smooth muscle cell division (Clark et al, 1984), a direct relaxation effect of testosterone on smooth muscle cells (Yue et al, 1995), and a change in the number of receptors for sympathetic affecter molecules (Colucci et al, 1982; Leipheimer and Sachs 1993). With regard to this latter possibility, it has been demonstrated that androgens (Leipheimer and Sachs 1993) as well as estrogens (Colucci et al, 1982) influence the sensitivity of smooth muscle cell α adrenergic receptors. It has also been reported that elderly men and men suffering from diabetes possess heightened sensitivity to α adrenergic agonists; men in these two groups also show a higher frequency of erectile dysfunction (Kaiser and Korenman 1988). Therefore, studies were performed to investigate if one of the mechanisms by which testosterone maintains erectile function is by altering the responsiveness of smooth muscle cells to adrenergic stimulation.

The following is the text of a manuscript entitled "Androgens Modulate the α-Adrenergic Responsiveness of Vascular Smooth Muscle in the Corpus Cavernosum."

This manuscript was published in the *Journal of Andrology* 1997: 18;26-31, and the authors included Christopher M. Reilly, Vivienne S. Stopper, and Thomas M. Mills.

ABSTRACT

Rat penile erection is an androgen dependent process with castration leading to a loss of potency. The present study was designed to determine if one of the mechanisms by which androgens maintain the erectile response is the regulation of the α adrenergic responsiveness of cavernosal smooth muscle. Electrical stimulation of the major pelvic ganglion (MPG) was used to elicit erection in untreated-castrated rats (CASTRATE) or castrated rats given testosterone replacement (TESTO). The effects of phenylephrine (an α_1-adrenergic agonist) and prazosin (an α_1 - adrenergic antagonist) on the erectile response were investigated. Phenylephrine, when administered to both TESTO and CASTRATE animals during erection, resulted in a dose dependent decrease in the intracavernosal pressure (CCP) with an ED_{50} value of 1.8 ± 0.48 µg/kg BW for TESTO rats; in the CASTRATE animals, the ED_{50} was significantly reduced to 0.29 ± 0.08 µg/kg BW. The increases in mean arterial pressure (MAP) resulting from phenylephrine injection in TESTO and CASTRATE animals were of similar magnitude and were not significantly different. Prazosin administration resulted in an enhancement of the erectile response in CASTRATE but not in TESTO animals. Taken together these results demonstrate that the cavernosal vasculature in CASTRATE animals possesses increased reactivity to α adrenergic stimulation as compared to the sensitivity in TESTO rats. Based on these findings we conclude that one of the mechanisms by which androgens maintain erectile function is by regulating the α_1 adrenergic responsiveness of the cavernosal smooth muscle.

Key words: penile erection, testosterone, adrenergic, prazosin, phenylephrine

INTRODUCTION

Penile erection results from a decrease in sympathetic tone of cavernosa smooth muscle coupled with the release of local vasodilators that lead to increased blood flow into the cavernous sinuses. Under the driving force of the mean arterial pressure (MAP) the sinuses fill with blood causing expansion of the cavernous sinuses and this expansion leads to compression of the cavernous veins which reduces outflow. The combined increase in inflow and decrease in outflow result in erection. Detumescence is produced by a re-establishment of sympathetic tone and a decline in the release of vasodilating agents. For recent reviews of this topic see (Burnett 1995; Andersson and Wagner 1995; Argiolas and Melis 1995).

Prior studies from this laboratory have demonstrated that rat penile erection is androgen dependent (Mills et al, 1996b; Mills et al, 1994; Mills et al, 1992). In other published reports, Heaton and Varrin, (1994), described a centrally mediated role for testosterone in the erectile response and Leipheimer and Sachs (1993), have demonstrated androgen sensitivity in the vascular muscle involved in erection. While it is widely held that androgens are responsible for erectile function both centrally and peripherally, the precise mechanisms of how androgens act to maintain the erectile process remains an enigma. Studies in castrated dogs have suggested that androgen deficiency could result in higher smooth muscle tone leading to incomplete trabecular smooth muscle relaxation (Muller et al, 1988). The present studies were designed to determine if rat cavernosal

smooth muscle tone is under androgenic regulation by testing the hypothesis that androgens modulate the adrenergic responsiveness of the vascular smooth muscle controlling blood flow in the penis.

MATERIALS AND METHODS

Animals: Six to nine months old male Harlan Sprague Dawley (Indianapolis, IN) retired breeders were used in these studies. The animals are housed in environmentally controlled quarters in separate hanging cages on a 14 h light: 10 h dark cycle and provided food and water ad libitum.

Castration and testosterone replacement: Animals were castrated under ether anesthesia and immediately implanted subcutaneously with a 3 mm (approx. 3 mg) pellet composed of cholesterol (group designation: CASTRATE) or 50% testosterone and 50% cholesterol (group designation: TESTO). Experimental procedures were performed 6 - 9 days after castration and pellet implantation. At termination of the experiment, blood from the carotid artery was collected and analyzed for circulating levels of testosterone by radioimmunoassay. Testosterone levels in CASTRATE rats averaged 80 ± 40 pg /ml, while TESTO animals had blood levels of 1050 ± 500 pg/ml. We have previously shown that this method of testosterone replacement fully restores the erectile function to that observed in an intact animal (Mills et al, 1992).

Preparation of animals for measurement of intracavernosal pressure: The method for stimulation and measurement of the erectile response is standardly used in this laboratory (Mills et al, 1996b; Mills et al, 1994; Mills et al, 1992). Briefly, the rat was anesthetized with an intramuscular injection of ketamine (87 mg/Kg) combined with xylazine (13 mg/kg) and maintained on supplemental injections of ketamine and xylazine as needed.

The carotid artery was cannulated with a blunted 18 gauge needle with PE 200 tubing attached to a pressure transducer and connected to a multichannel recorder to continuously monitor mean arterial blood pressure (MAP). Via the femoral artery, a fine tipped cannula was inserted into the dorsal aorta for drug delivery. The abdominal cavity was next opened, the viscera retracted, covered in saline soaked sponges and wrapped in cellophane to reduce evaporation and to help maintain body temperature. The tissue overlying the right aspects of the dorsal prostate was cleared of fat and adhering fascia to expose the right cavernosal nerve and major pelvic ganglion (MPG). The shaft of the penis was cleared of the overlying fascia and the right corpus cavernosum was exposed. The cavernosum was cannulated with insertion of a 30 gauge needle which was attached to PE 200 tubing (drawn to a fine tip) and attached to a pressure transducer for measurement of intracavernosal pressure (CCP).

Measurement of the Erectile Response: Stainless steel bipolar electrodes connected to a Grass stimulator, were positioned on the pelvic nerve and the ganglion using a micro manipulator. Proper electrode placement was established by determining the position of electrodes that produced the maximal CCP with minimal alteration of MAP at a 5 volt stimulation (pulse duration -5 msec, frequency -12 Hz.). After electrode placement, each animal was subjected to a series of stimulations of the MPG ranging from 1 to 6 volts while CCP and MAP were continuously measured. Stimulations lasted one minute with a recovery time of one minute between stimulations. Previous studies from this laboratory have determined that 5 or 6 volts gives the maximal erectile response (Mills et al, 1992).

Administration of Phenylephrine During induced Erection: After determining the optimal voltage and electrode placement, the effects of intra-aortic administration of phenylephrine on intracavernosal pressure was determined. In these experiments, the CCP was allowed to rise to a maximum (after about 30 second) and then phenylephrine was administered into the aorta in a single bolus in doses of 0.01, 0.05, 0.1, 0.5, 1.0, 5.0, 10.0, and 50.0 μg/kg body weight (BW) in 250 μl of saline. An equal volume of saline was administered as a control. Stimulation was continued for a total of 2 min followed by a 2 - 3 min recovery between phenylephrine doses.

Administration of Prazosin: Since prazosin is sparingly soluble in saline, it was dissolved in distilled water at a concentration of 1 mg/ml. After a control injection (intra-aortic) of 250 μl of water into the dorsal aorta followed by a 5 min equilibration period, the animal was subjected to sequential stimulatory voltages from 1 - 6 volts for one minute with a recovery time of 1 minute between stimulations (PRE-PRAZOSIN). Five-hundred μg prazosin/kg BW was then injected in 250 μl water and, 5 minutes after prazosin injection, the series of stimulation of the MPG (1 - 6 volts) was repeated (POST-PRAZOSIN). To determine if the rate at which blood drained out of the cavernous sinuses was altered by prazosin treatment, the intracavernosal pressure at the time that the ganglionic stimulation was terminated was compared to the intracavernosal pressure 12 seconds later. This comparison was made in the PRE-PRAZOSIN and POST-PRAZOSIN animals in both treatment groups.

Statistical Analysis: To analyze the results of the phenylephrine injection experiment, polynomial regression was used to determine the best fit through the data points and the resulting regression lines were used to determine the ED_{50} value for the drug in each animal. The ED_{50} values for TESTO and CASTRATE animals were then compared by students t-test. To compare the effects of prazosin on the erectile response in TESTO and CASTRATE rats, repeated measures ANOVA was employed with post hoc analysis by the method of least squares. The rate of outflow was analyzed statistically using repeated measures ANOVA followed by post hoc analysis with Dunnett's test (Winer 1971). $P<$ 0.05 was considered statistically significant.

RESULTS

Figure 1 shows a representative tracing of intracavernosal pressure (CCP) and mean arterial pressure (MAP) responses to stimulation of the MPG in a TESTO and CASTRATE rat. After the CCP had reached a maximum, phenylephrine was injected causing a rapid decline in CCP but an increase in MAP. While the MAP values increase to about the same extent in the TESTO and CASTRATE animals, this tracing shows three differences between the CCP response in CASTRATE rats and TESTO animals. The first difference is the magnitude of the erectile response. The CCP increase relative to the MAP rise (CCP/MAP ratio) in CASTRATE animals is about 0.3 while the ratio in TESTO animals is about 0.6. Secondly, the magnitude of the decline in the CCP/MAP ratio resulting from administration of an equal dose of phenylephrine is greater in CASTRATE rats than in TESTO animals. In this tracing at a dose of 1 µg phenylephrine/kg BW, the CCP in the TESTO animal was reduced by about 50 %, whereas in a CASTRATE animal at the same dosage, the CCP was decreased by nearly 90%. Thirdly, the degree of recovery of the CCP after drug administration in CASTRATE animals is less than the recovery in TESTO animals in which the CCP returns and often overshoots the original pressure. These differences suggest that the response of the cavernosal smooth muscle in TESTO and CASTRATE animals is not the same.

Analysis of the effects of phenylephrine on CCP is shown in Figure 2. The maximal intracavernosal pressure decrease following phenylephrine administration was set to 100% and the responses to other doses of the drug expressed as percent of this maximum. Figure 2 shows a dose dependent decline in the CCP in both groups with

Figure 1. Representative tracings of intracavernosal pressure (CCP) and mean arterial pressure (MAP) responses to ganglionic stimulation (solid horizontal bar) at 5 volts and injection of 1 μg phenylephrine/kg BW ( ) into the aorta of a CASTRATE and TESTO animal.
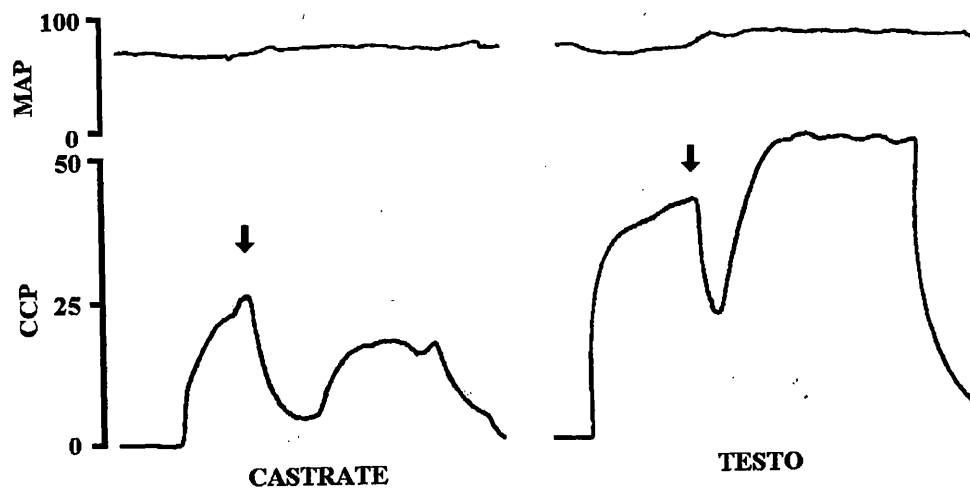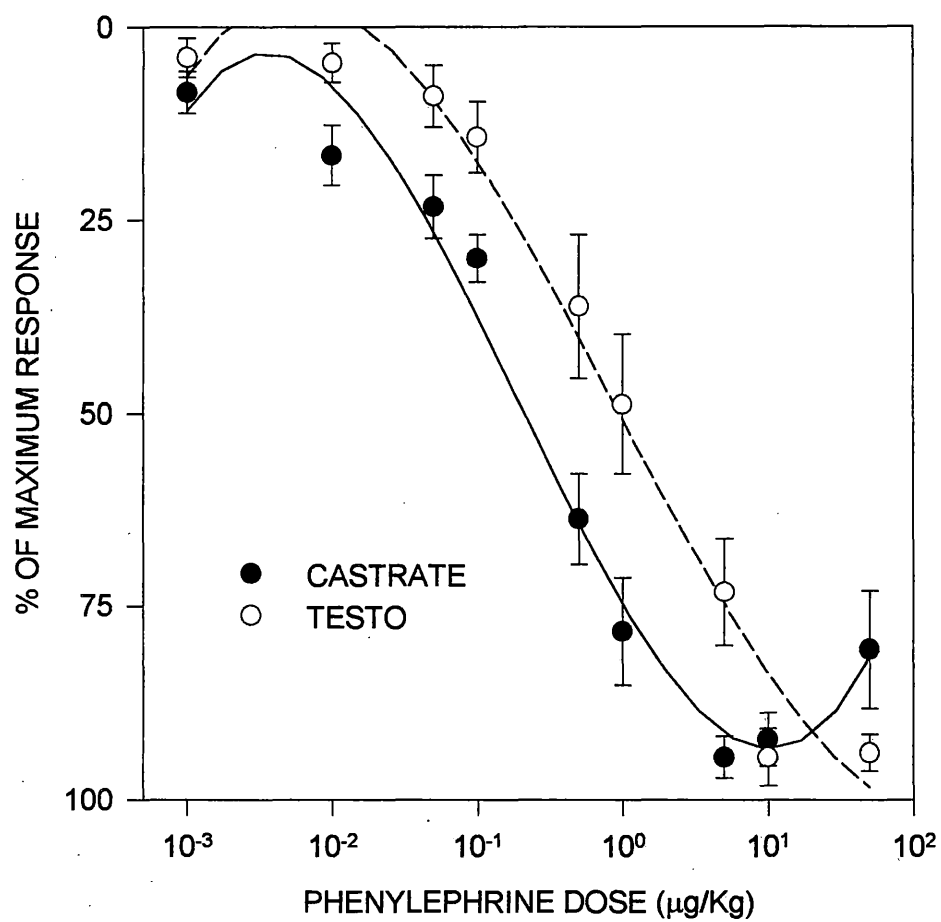
MAP

CCP

100

0

50

25

0

CASTRATE

TESTO

Figure 2. The effects of increasing doses of phenylephrine on the intracavernosal pressure

(CCP) during electrical stimulation of the major pelvic ganglion in CASTRATE and

TESTO animals. Each point is the mean ± SEM of measurements made in 7 -11 animals.

administration of increasing amounts of phenylephrine and also shows a greater

responsiveness in CASTRATE animals than in TESTO rats. Further analysis of the results

with the determination of the ED_{50} for the response (the dose of phenylephrine which

causes a 50% reduction in CCP) reveals that the CASTRATE animals are more responsive

to the drug than the TESTO rats. Our calculations show that the ED_{50} for CASTRATE

animals was 0.29 ± 0.08 µg/kg BW, whereas the ED_{50} value in TESTO animals was

significantly increased to 1.8 ± 0.48 µg/kg BW. This shows that the responsiveness to

phenylephrine is nearly 6 times greater in CASTRATE animals than in the TESTO rats.

To establish that the differences in phenylephrine sensitivity was specific to the

corpus cavernosum and not due to generalized effects acting on the systemic vasculature,

changes in MAP following drug administration were measured (Figure 3). These results

demonstrate that the dose dependent increase in the MAP following phenylephrine in

CASTRATE animals is not different from the increase in TESTO animals.

To confirm that CASTRATE animals are more responsive to α adrenergic agonists

than rats in the TESTO group, studies were completed to determine if the sensitivity of

the corpus cavernosum to prazosin, an α_1 adrenergic antagonist, was different in the two

treatment groups. Table 1 shows that in both the CASTRATE and TESTO groups, the

MAP decreased immediately following prazosin injection and remained low for the

duration of the experiment. Figure 4 demonstrates the effect of prazosin administration on

the erectile response (CCP/MAP) in CASTRATE and TESTO animals and shows that

there was an overall significant enhancement in the erectile response (CCP/MAP) in

CASTRATE animals (PRE-PRAZOSIN vs. POST-PRAZOSIN) but not in the TESTO

Figure 3. The effects of increasing doses of phenylephrine on the mean arterial pressure (MAP) during electrical stimulation of the major pelvic ganglion in CASTRATE and TESTO rats. Each point is the mean ± SEM of measurements made in 7 - 11 animals. The responses in the two treatment groups are not significantly different.
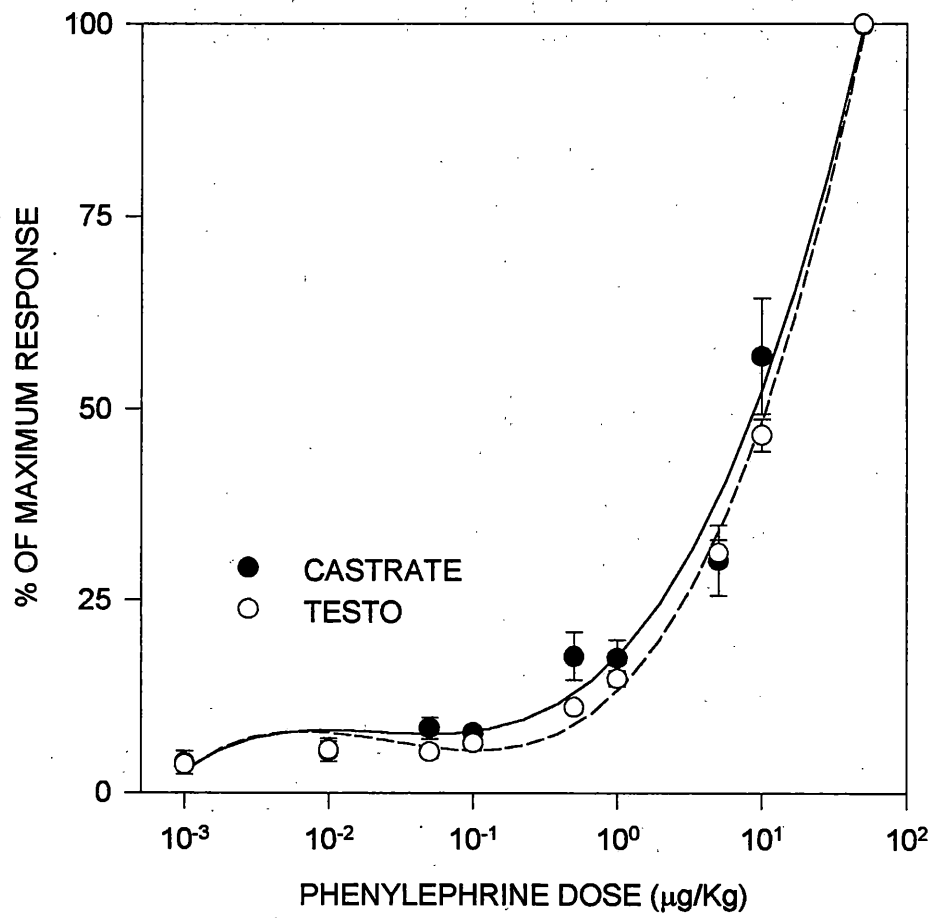
Table 1. Effects of the duration of prazosin treatment (0-15 minutes) on MAP (mmHg) in

CASTRATE and TESTO animals.*

Treatment	0 minutes	1 minutes	5 minutes	10 minutes	15 minutes
CASTRATE	81±6	67±7	66±5	66±3	68±2
TESTO	82±5	71±3	70±3	69±4	70±5

* Each value is the mean of measurements made in 4 - 5 animals ± 1 SEM. The MAP

following prazosin administration is significantly different from that of pre-injection (0

minutes) at all time observed ($P < 0.05$). However, there was no significant difference

between the mean blood pressure values in the CASTRATE and TESTO animals at any of

the specific times ($P > 0.05$).

rats (Figure 4).

Following prazosin administration, the rate at which blood drained from the corpus cavernosum was significantly altered in both treatment groups. Figure 5 shows that when ganglionic stimulation (3 - 6 volts) was discontinued, drainage from the cavernous sinuses was significantly decreased in both CASTRATE and TESTO animals but the rates of drainage were not different in the two treatment groups.

Figure 4. Effects of stimulation of the major pelvic ganglion in CASTRATE and TESTO

animals before and after prazosin administration (PRE-PRAZOSIN and

POST-PRAZOSIN). Each value is the mean $\pm$ SEM of measurements made in 4 - 5

animals. At 5 and 6 volts the CCP/MAP ratio is significantly greater in the CASTRATE

group after receiving prazosin ($P < 0.05$), while the TESTO group remains unchanged.

Figure 5. Effects of prazosin administration on the rate of drainage in the corpora

cavernosa after stimulation in CASTRATE and TESTO animals. Each value is the mean

± SEM of measurements made in 4 -5 animals. The rate of drainage in both CASTRATE

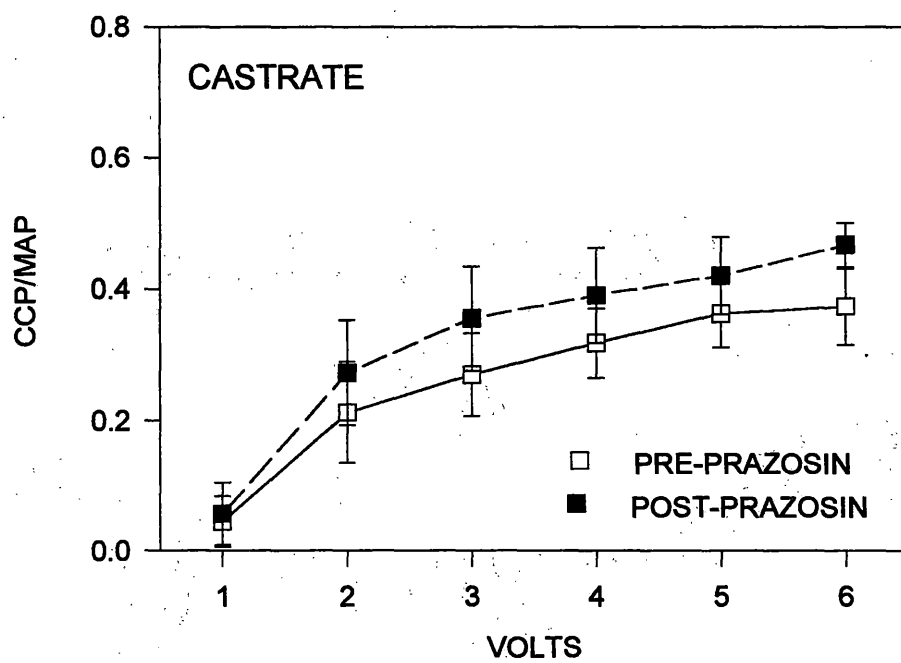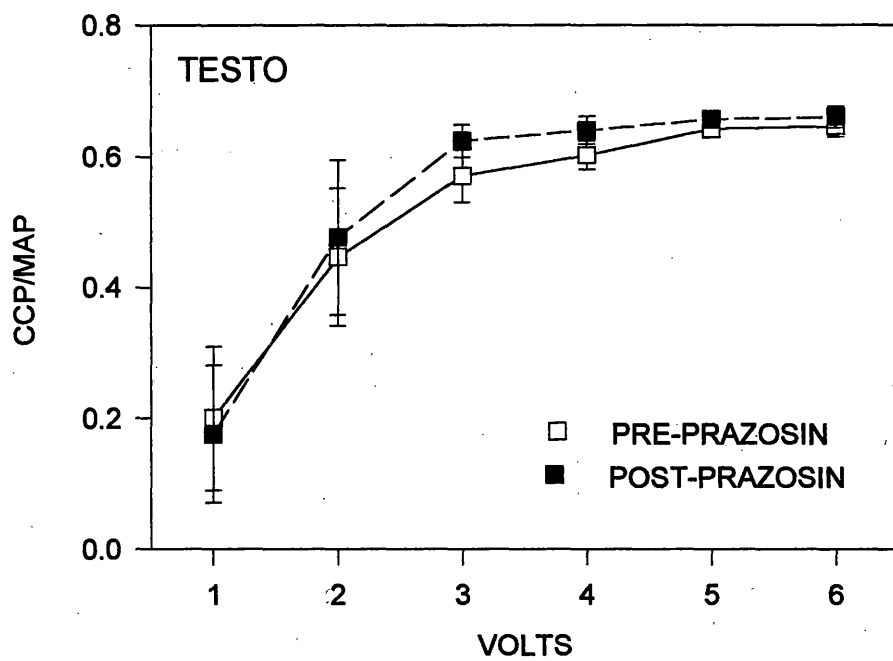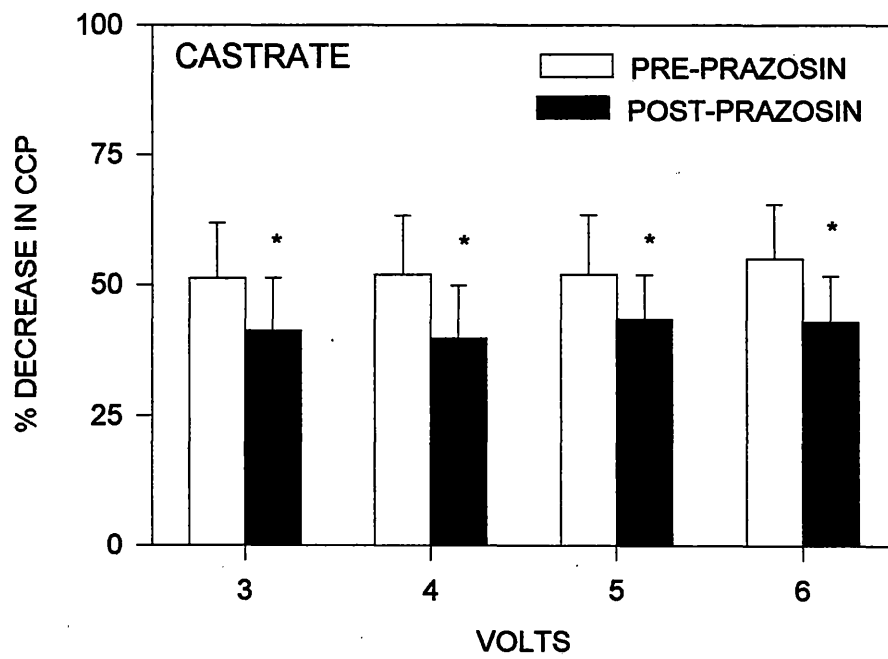and TESTO animals is significantly decreased with prazosin administration (*P <0.05)

after 3-, 4-, 5-, or 6- volt stimulation.
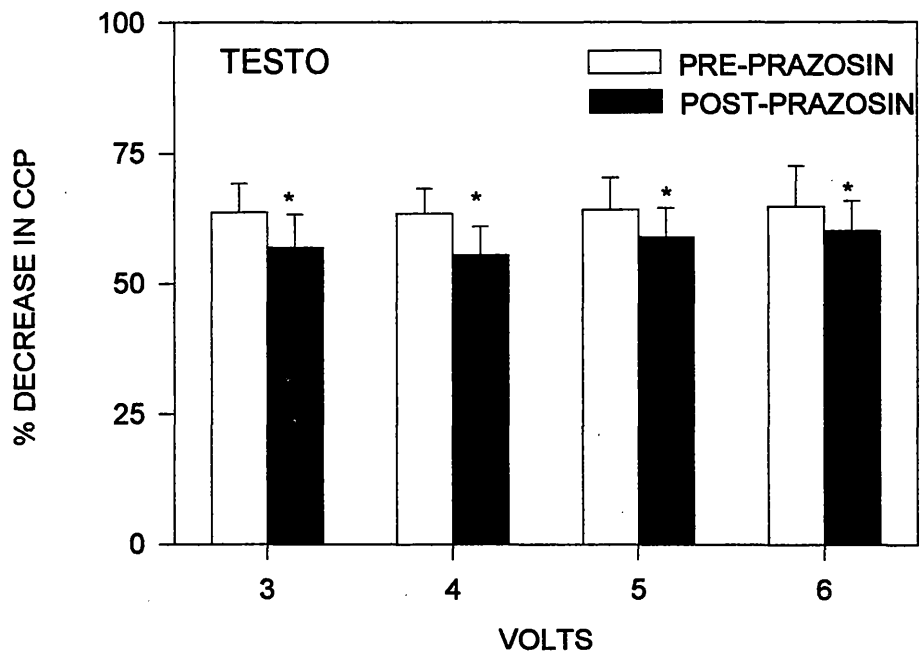
DISCUSSION

While the role of the muscarinic cholinergic system in the erectile response remains controversial, the adrenergic system has been shown to be critically important. Both α and β adrenergic receptors have been identified in the corpus cavernosum, with α receptors outnumbering β receptors 10 to 1 (Christ et al, 1990; Levin and Wein 1980). Systemic administration of β adrenergic antagonists either failed to affect, or in some instances inhibited, penile function. On the other hand, α adrenergic antagonists substantially enhanced erectile function when used in conjunction with non-adrenergic non-cholinergic vasodilators (Truss et al, 1994; Levin et al, 1994; Adaikan and Ratnam 1988; Blum et al, 1985; Wagner and Brindly 1980). Specifically a tri-injection cocktail containing papaverine and PGE_1 (to facilitate vasodilation) and phentolamine (to decrease sympathetic tone) has been used successfully to elicit erection sufficient for intercourse in otherwise impotent men. In other clinical studies, intracavernous injection of the α_1 adrenergic antagonist, prazosin (Christ et al, 1992; Holmquist et al, 1990; Blum et al, 1985) or the α_1, α_2 adrenergic antagonist, phentolamine (Diederichs and Lue 1991) resulted in an increase in CCP and partial erection. Idazoxin (an α_2 antagonist) has little effect suggesting that the α_1 subtype is the predominant receptor involved (Argiolas and Melis 1995). In cases of priapism, injection of phenylephrine (an α_1 agonist) into the corpus cavernosum is often used to re-establish detumescence (Dittrich et al, 1991). Taken together these reports confirm that elevated sympathetic tone maintains detumescence and the necessity for a decrease in sympathetic tone for erection to occur. Furthermore, the target of α adrenergic agonists in the regulation of cavernosal blood

flow would have to be the smooth muscle of the cavernosal arterioles since arteriolar flow

controls organ blood flow (Berne and Levy, 1992).

Previous studies from this laboratory have demonstrated that the magnitude of the

erectile response in castrated animals receiving testosterone replacement is similar to that

of intact animals, whereas untreated-castrate animals display a significant decrease in the

erectile response (Mills et al, 1996b; Mills et al, 1994; Mills et al, 1992). The present

studies were undertaken to investigate the underlying mechanisms by which androgens act

to maintain the erectile response. The results in Figure 2 show that cavernosal smooth

muscle from CASTRATE rats is nearly 6 times more responsive to phenylephrine than

smooth muscle from TESTO rats. However, it is not apparent from these findings how

the loss of testosterone leads to the increase in responsiveness to the α agonist. There are

several possible mechanisms by which the androgen could exert this action: Zhang and

coworkers (1991), suggested steroid hormones could regulate anion metabolism and Ca^{2+}

transport across smooth muscle cell membranes. Other possible mechanisms include

androgenic regulation of smooth muscle cell division (Fujimoto et al, 1994), a direct

relaxation effect of testosterone on smooth muscle cells (Yue et al, 1995), or a change in

the number of receptors for sympathetic affecter molecules. With regard to this latter

possibility, it is known that androgen (Leipheimer and Sachs 1993), as well as estrogens

(Colucci et al, 1982), influence the sensitivity of smooth muscle cell adrenergic receptors.

Also reported was the finding that elderly men and men suffering from diabetes, possess

heightened sensitivity to α adrenergic agonists; men in these two groups also show a

higher frequency of erectile dysfunction (Christ et al, 1992). We suggest that testosterone

could regulate the number of α receptors either by down regulation of receptor synthesis or by increasing the rate of receptor turnover although there is no evidence in the present report to support either possibility. Normally in the corpus cavernosum, the number of α receptors outnumbers the number of β receptors by 10 to 1 (Levin and Wein 1980) and the number of β receptors in smooth muscle decreases with age (Hishmoto et al, 1995). The loss of testosterone support could alter the ratio of α and β adrenergic receptors and thereby alter the sympathetic tone of the tissue. If castration leads to a decline in the number of smooth muscle cells in the corpora cavernosa, then a reduction in cell number would limit the amount of NO produced resulting in a reduced erectile response.

The quantity of prazosin injected into the animal was similar to the dose used by Waeber et al, (1983), who showed that this dose of the α adrenergic antagonist caused a 15 -20 mm Hg decrease in MAP for a period of 30 - 45 min. Studies from this and other laboratories reported that when MAP is increased or decreased during erection, CCP changes proportionally (Mills et al, 1994; Sachs and Liu, 1992). We find that after prazosin injection, the response to ganglionic stimulation changed little in TESTO animals whereas the response increased significantly in CASTRATE rats. This finding, in conjunction with the findings of other investigators, shows that the suppression of sympathetic tone is essential to full erectile function (Giuliano et al, 1993a; Costa et al, 1993; Clark et al, 1988; Diederichs and Lue, 1991).

Following the cessation of ganglionic stimulation of prazosin treated rats, blood drained from the penis at a decreased rate in both CASTRATE and TESTO animals. Thus, the sympathetic tone of cavernosa smooth muscle also influences the rate of

drainage of blood from the penis. Although we could not establish a direct relationship between androgen administration and the rate of outflow, it is evident that sympathetic activity is involved. Possibly, the prazosin treatment slowed the rate at which sympathetic tone was re-established in the cavernosal arteries. This could result in continued high rates of flow of blood into the cavernous sinuses to partially maintain the veno-occlusive mechanism and slow the rate of drainage. Alternatively, the findings could reflect a direct action of phenylephrine on the vaso-occlusive mechanisms although this seems unlikely based on our published findings detailing the unresponsive nature of the veno-occlusive mechanism to vasoactive drugs (Mills et al, 1994).

In summary, these studies suggest that sympathetic activity in the corpus cavernosum is regulated, in part, by testosterone. Testosterone acts to reduce the responsiveness to α_1 adrenergic agonists and the reduced sympathetic tone permits greater blood inflow and higher intracavernosal pressure.

CHAPTER 5

SUMMARY AND CONCLUSIONS

In recent years there have been many advances in the understanding of erectile function. Based on these advances, it can be concluded that a balance between smooth muscle relaxation and contraction is required for normal erectile function. The results from clinical and experimental studies indicate that a critical degree of smooth muscle relaxation is required to convert the tonically contracted flaccid penis to the erect state. A considerable amount of data have demonstrated that NO acts as the principal mediator of penile erection. NO is produced and released primarily by penile nerves and this neurotransmitter diffuses locally to stimulate smooth muscle relaxation by a cGMP dependent mechanism. Evidence also suggests that the release of NO by sinusoidal endothelium may contribute to relaxation of the cavernosal smooth muscle. Although NO has been determined to be the predominant vasodilatory neurotransmitter involved in erectile function there are other pathways that can be initiated to elicit an erectile response. The possible interaction of these other vasodilatory neurotransmitters is still poorly characterized and their role in erectile function and physiologic importance remains controversial despite the fact that other peptides often exert potent effects on penile tissue.

With regard to tissue contractility, norepinephrine binding to post-junctional α_1 adrenergic receptors on cavernosal smooth muscle cells is the primary mediator of detumescence. Therefore, a decrease in sympathetic tone is required for vasodilation. In

addition to norepinephrine release resulting in smooth muscle contraction, corporal

endothelial cells have been shown to synthesize and release endothelin, a potent

vasoconstrictor of cavernosal smooth muscle cells; endothelin-1 may also be partially

responsible for flaccidity of the penis. Depicted in Figure 1 is a schematic representation

of how these neurotransmitters are thought to control the state of contraction and

relaxation of cavernosal smooth muscle during erection.

Testosterone plays a critical role in maintaining erectile function in rats.

Traditionally, androgens have been thought to mediate only central nervous system

processes allowing erectile function. More recently, it has become apparent that

androgens influence penile tissue directly. The objective of this study was to determine

how androgens maintain erectile function in peripheral tissue using castrated male rats

with or without testosterone replacement.

In the first set of studies, the importance of NO in mediating the erectile function

was demonstrated. By using inhibitors of NO as well as NO donating drugs these studies

confirmed that the major vasodilating neurotransmitter in penile tissue is NO. These

studies also demonstrated that in castrated rats, the amount of nNOS mRNA was

decreased by 50% as compared to castrated rats receiving testosterone replacement.

These studies together with others show that androgens enable erectile function, in part,

by maintaining nNOS gene expression or by suppressing the degradation of nNOS mRNA.

The second set of experiments were designed to determine if androgens could also

support erectile function by biochemical pathways which do not include NO synthesis.

L-NAME was administered chronically to abolish all endogenous NO production in these

animals and partial erectile responsiveness was demonstrated in testosterone supplemented castrated rats. Furthermore, though the use of stimulators and inhibitors of the soluble guanylate cyclase enzyme, testosterone was found to enhance guanylate cyclase activity.

In the third study, the actions of testosterone on sympathetic tone in cavernosal tissue was investigated. In these experiments an α adrenergic agonist and an α_1 adrenergic antagonist were injected into the aorta or corpora cavernosa of castrate and castrate rats supplemented with testosterone: Intracavernosal pressure was measured to determine if testosterone altered the tissue sensitivity to these vasoactive drugs. These results suggest that testosterone decreases the sensitivity to α adrenergic stimulation.

Thus, it can be concluded that testosterone acts to maintain the erectile response in the rat penis in a variety of ways. Androgens act to increase the availability of NO, as well as mediate other non NO pathways and support the sensitivity to α adrenergic agonists. The combination of the increased action of vasodilatory neurotransmitters along with the androgen mediated decrease in contractile stimulation leads to a greater increase in intracavernosal pressure. The multiple sites of androgenic action exemplify how multiple physiological processes are activated which lead to the erectile response in the penis.

Figure 1. Schematic representation of the neuronal control of erectile function.

NANC- non-adrenergic non-cholinergic

NE- norepinephrine

ACh- acetylcholine

NO- nitric oxide

nNOS- neuronal nitric oxide synthase
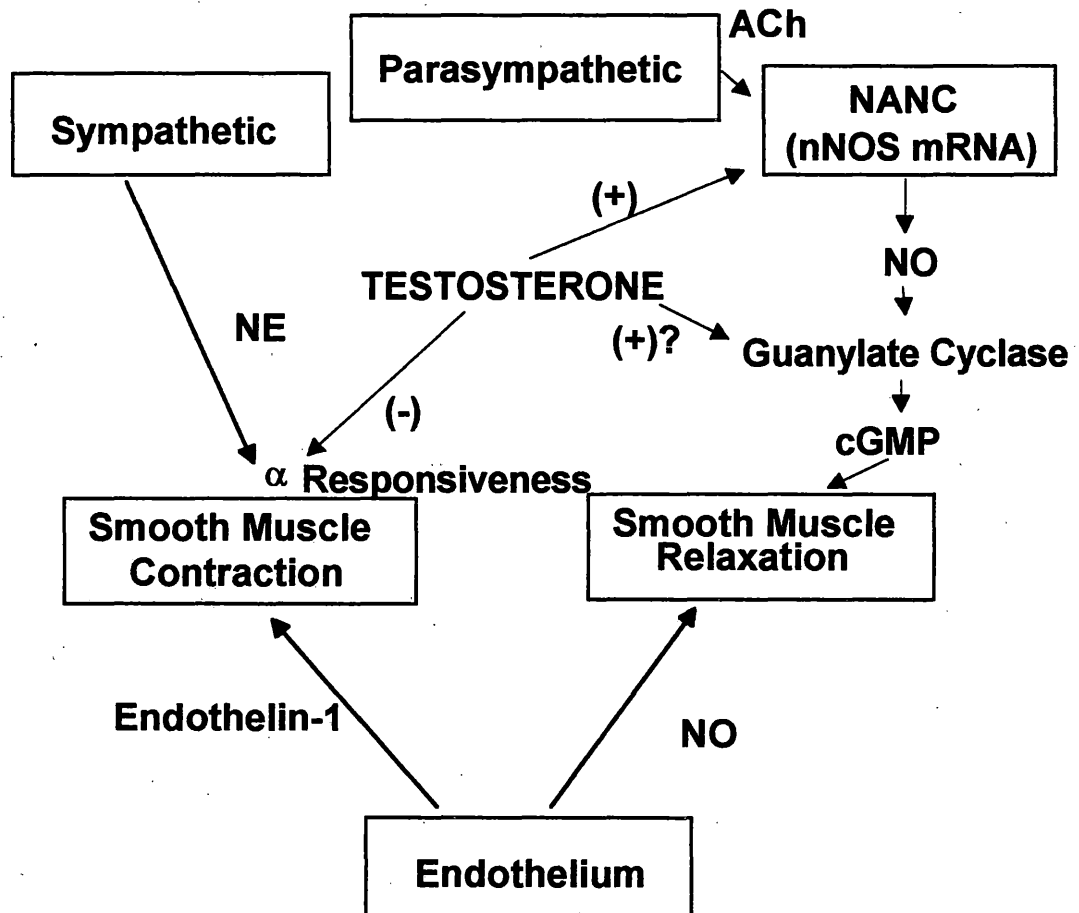
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