The Effects of Castration on Relaxation of Rat Corpus Cavernosum Smooth Muscle *In Vitro*

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Submitted in Partial Fulfillment of the Requirements

for the Degree of

Master of Science

in the

Biological Sciences

Program

YOUNGSTOWN STATE UNIVERSITY

August, 1998

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ABSTRACT

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Purpose : This study was conducted to investigate the role of testosterone in regulating the relaxation of isolated rat corpus cavernosum strips *in vitro*.

Materials and Methods : Male rats were divided into treatment groups of intact, castrate, and castrate with testosterone replacement. Norepinephrine was added to contract each of the tissue strips. Next, sodium nitroprusside, experiment I, or 8-bromo-cGMP, experiment II, was added to relax the cavernosum tissue. Percent relaxations were recorded for each treatment group at each dose level.

Results : Sodium nitroprusside was added to the norepinephrine contracted tissues in doses of 10^{-4} and 10^{-3} M. In this experiment, castration significantly reduced tissue responsiveness to sodium

nitroprusside and testosterone replacement restored the response to intact levels. In the second experiment 8-bromo-cGMP was added to the norepinephrine contracted tissues in doses of 10^{-5} and 10^{-4} M. 8-bromo-cGMP 10^{-4} M was significantly less effective in relaxing tissue from castrate animals as compared to intact controls. Again, testosterone treatment restored the response to intact levels.

Conclusions : Our results show a clear role of testosterone in regulating the ability of corpus cavernosum tissue to relax when treated with sodium nitroprusside or 8-bromo-cGMP in vitro. In addition, the data suggests that testosterone regulates sites distal to the formation of cGMP in this smooth muscle relaxation pathway.

ACKNOWLEDGMENTS

My utmost appreciation to:

--- Dr. Robert Leipheimer

--- Dr. James Toepfer

--- Dr. Gary Walker

--- David Bloomberg

--- Tonya Frost

--- Michael Arvin

--- Lenka Fedorkova

--- Paula Capobianco

Without whom I would have never been able to complete my work.

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Introduction

Impotence is a common disease observed worldwide among men. This disorder affects nearly 10 million men in the United Sates alone (Ignarro, 1992). Impotence has also been demonstrated to be age dependent, increasing in frequency in the elderly. The incidence of the disease increases from 2% in men age 40 to 25% in men age 65 (Ignarro, 1992) and to 80% in men age 80 (DePalma, 1996). The failure to develop tumescence has been linked to several physiologic mechanisms. Changes in arterial blood supply, venous resistance, and smooth muscle relaxation have all been implicated in the onset of physiologic impotence (Lue & Tanagho, 1987). Generally impotence occurs when these mechanisms are modified due to loss of blood flow into the corpora, a failure to develop venous occlusion to prevent blood efflux, and a lack of smooth muscle cell function. Decreased blood flow has been shown to be the most common cause of impotence in men, whereas endocrine associated impotence contributes only 3-4% of reported cases (DePalma, 1996).

Many pharmacological treatments have been developed in order to restore erectile function. Early treatments favored corporal vascularization surgeries (DePalma, 1996). More recently both topical and injectable drugs have been shown to be effective in producing tumescence in man and animal trials (review in Lue & Tanagho, 1987). Vasoactive drugs such as phentolamine and papaverine have been used commonly in human trials to treat impotence (Lue & Tanagho, 1987). However, difficulty in direct penile injection of these chemicals in man has led to the development of topical treatments. Topical agents such as stearyl - vasoactive intestinal polypeptide, a fat-soluble analogue of vasoactive intestinal polypeptide, have been shown

to increase sexual activity in rats (Gozes & Fridkin, 1992). In addition, oral drugs such as the α–adrenergic blockers isoxsuprine hydrochloride and yohimbine hydrochloride have been effective (DePalma, 1996). More recently, the phosphodiesterase inhibitor sildenafil, brand name viagra, has been used successfully to restore erectile function (Goldstein *et al.,* 1998). In many cases over the last twenty years, penile implants have been used to treat erectile dysfunction. Over 250,000 men have received prostheses of some variety, however only 71% are satisfied with the device (DePalma, 1996).

Unfortunately, our lack of complete understanding of the physiologic mechanisms involved in erection has limited treatment effectiveness. In addition the origin of erection in humans is not well defined. There is still great debate over the difference between psychogenic erection, those resulting from sensory input to the cortex, and reflexogenic erection , those resulting from direct genital stimulation (Sachs, 1995). Furthermore, the biochemical pathways involved remain unclear. To begin to study the physiology behind tumescence, a thorough review of penile anatomy is necessary.

The erectile portion of the penis consists of the corpus spongiosum, which lies medially in the penis and contains the urethra, and the corpus cavernosum, a pair of two tissue bodies each lying lateral to the corpus spongiosum. The erectile bodies consist of primarily smooth muscle arranged among cavernous spaces by connective tissue. Each group of erectile tissue is linked with striated muscles. The bulbocavernosus attaches to the penile bulb at the end of the corpus spongiosum, while the ischiocavernosus is continuous with the corpus cavernosum (de Groat & Booth, 1991). These erectile bodies along with their associated skeletal

muscles play a key role in erectile function and impotence.

The corpora of the penis are surrounded by the fibrous tunica albuginea. This has been shown in man to be a bilayered structure consisting of elastic and collagen fibers (Brock *et al.*, 1997). The tunica runs continuous with the vas deferens and testes. The intact tunica is necessary for proper corporal filling with blood. Penile blood flow is supplied through penile arteries, which enter the corpora and branch into numerous helical arteries. Venous drainage occurs through several deep veins entering the central penile vein (de Groat & Booth, 1991). In the rat, these cavernous veins form parallel channels along the base of the penis (Mills *et al.*, 1996). The vasculature and tunica are each associated directly with the development of tumescence. At the onset of erection, blood fills the corpora, expanding them against the tough tunica providing the venous occlusion required for erection (Mills *et al.*, 1996).

The penile tissue is innervated directly by peripheral neurons of the major pelvic ganglion. In man, the perineal and dorsal penile nerves, branches of the pudendal nerve directly innervate the cavernous tissue. These fibers contain both cholinergic and adrenergic neurons, as well as non-adrenergic non-cholinergic neurons (de Groat, 1993). In the rat, the major nerve innervating the penis has been termed the cavernous nerve (Quinlan *et al.*, 1989). Development of tumescence is directly controlled by the nervous system through these innervating neurons.

Rats have been commonly used in the study of penile erection due to reproducibility of the erectile response and low cost (Quinlan *et al.*, 1989). In addition, the rat model is particularly useful due to the lack of adrenal gland androgen production (Mills *et al.*, 1996). One week after castration, levels of dehydroepiandrosterone, dihydrotestosterone, and testosterone are

undetectable. This is quite different from the human model, in which the adrenal cortex produces adequate androgen quantities to maintain erection (Mills *et al.*, 1996). Both canine and rabbit models have also been commonly used with various amounts of similarity in results to that in the rat and human.

Each of the aforementioned anatomical components plays a key role in the erectile process, and therefore, are vital to the physiologic pathways that lead to tumescence. At the onset of erection, increased arterial blood inflow into the penile corpora is observed (Newman & Northup, 1981). This influx of blood into the cavernous spaces and subsequent smooth muscle relaxation leads to the swelling of the penile tissue causing erection. In addition, the inflow of blood is accompanied by venous occlusion to prevent blood efflux. In the canine, both intrinsic and extrinsic penile occlusion mechanisms exist (Matsuzaka *et al.*, 1996). Poor venous occlusion has been implicated in the majority of physiologic erectile dysfunction (Lugg *et al.*, 1995a). The striated muscles of the penis have been shown to exert a role in controlling intensity of erection and ejaculation in the rat (Sachs, 1982). Excision of these muscles results in infertility in the rat. Absence of the ischiocavernosus led to a lack of penile dorsiflexion needed for intromission, and absence of the bulbiocavernosus led to the loss of intense erection (Sachs, 1982).

Neural control of penile tone is carried out through adrenergic neurons. Smooth muscle contraction during detumescence is mediated via adrenergic fibers in the penis. Norepinephrine has been shown to contract corpus cavernosum smooth muscle *in vitro* in the rat (Dail *et al.*, 1987). During periods of penile flaccidity, norepinephrine release from adrenergic neurons predominates. The contractile process has been shown to be

mediated by binding of α-adrenergic receptors and is blocked by the α-adrenoreceptor antagonist phentolamine (Dail *et al.*, 1987). This adrenergic activity is regulated by cholinergic neurons in the penis. Acetylcholine release has been shown to decrease norepinephrine release through binding of prejunctional muscarinic receptors (Saenz de Tejada *et al.*, 1989). In addition, the nonadrenergic non-cholinergic neurotransmitter vasoactive intestinal polypeptide does not appear to regulate the adrenergic nerves (Saenz de Tejada *et al.*, 1989). Besides norepinephrine, several other chemicals may modulate penile tone, such as prostanoids, endothelins, neuropeptide Y, and arginine vasopressin (review in Andersson & Holmquist, 1994).

Neurologic control of the erectile process appears to be mediated primarily through relaxation of the cavernous smooth muscle by stimulation from non-adrenergic non-cholinergic neurons (review in Lugg *et al.*, 1995a). The primary neurotransmitter involved has been identified as nitric oxide, previously known as endothelium derived relaxing factor (Ignarro, 1992). In addition, many other neurotransmitters have been related to the erectile process, however their roles remain less clear (Burnett, 1995). These chemicals include vasoactive intestinal polypeptide and related peptides, calcitonin gene-related peptide, prostaglandin, and adenosine triphosphate (review in Andersson & Holmquist, 1994). Calcitonin gene-related peptide has a direct effect on cavernous tissue through interaction with nitric oxide (Lugg *et al.*, 1995a). Furthermore, additional transmitters such as substance P and histamine have been shown to have both relaxant and contractile effects on cavernous tissue (review in Andersson & Holmquist, 1994). Nitric oxide has been demonstrated as the mediator of corpus cavernosum relaxation in man (Rajfer *et al.*, 1992; Kim *et al.*, 1991), dog (Trigo-Rocha

et al., 1993a), rat (Ignarro, 1992), and rabbit (Trigo-Rocha *et al.*, 1993b). Nitric oxide is produced by the enzyme nitric oxide synthase. Application of N-monomethyl-L-arginine or N-nitro-L-arginine, nitric oxide synthase inhibitors, obliterates the nitric oxide induced relaxation (Kim *et al.*, 1991) and (Burnett, 1995). Whether the nitric oxide synthase is restricted to the nerve terminals or located in the cavernosum smooth muscle has yet to be determined.

Due to the uncertainty of the location of nitric oxide synthase, the role of an intact endothelium has come into question. Direct stimulation of vascular smooth muscle relaxation by nitric oxide does not appear to be endothelium dependent (Liu *et al.*, 1992). However, in rabbit cavernosum tissue, erectile response to electric field stimulation is impaired by endothelium denudation (Kim *et al.*, 1994). In addition, non-adrenergic non-cholinergic stimulation is inhibited by endothelium removal (Liu *et al.*, 1992). In contrast, the relaxant effects of cholinergic neurons are well known to be endothelium dependent. The effects of acetylcholine are greatly inhibited by endothelium denudation in man (Kim *et al.*, 1991) and canine (Trigo-Rocha *et al.*, 1993b). Although, the response to electrical field stimulation was less inhibited by endothelium removal (Trigo-Rocha *et al.*, 1993b) reinforcing the fact that nitric oxide is the primary neurotransmitter involved. Nonetheless, the endothelium appears to play a role in penile erection, particularly in mediating the effects of acetylcholine.

As was previously mentioned, acetylcholine has been shown to decrease adrenergic tone in the penis. This effect along with others leads to a clear role for acetylcholine in the erectile process. Direct application of acetylcholine has produced mixed results. In the rat (Dail *et al.*, 1987), rabbit (Seftel *et al.*, 1996), and canine (Yeh *et al.*, 1994) direct addition of

acetylcholine has led to corpus cavernosum smooth muscle relaxation. However, it has not resulted in production of full erection *in vivo* (Suh *et al.*, 1995). The mechanism by which acetylcholine relaxes the corpora is mediated by nitric oxide production. Addition of N-monomethyl-L-arginine blocks the acetylcholine-induced relaxation (Seftel *et al.*, 1996). The nitric oxide synthase responsible for producing nitric oxide may be located in the endothelium leading to its dependence in this response. Acetylcholine also appears to modulate the effects of other relaxing neurotransmitters in the penis. For example, addition of acetylcholine and vasoactive intestinal polypeptide acted synergistically in relaxing canine corpus cavernosum (Takahashi *et al.*, 1992).

Despite the debate over which neurotransmitter is most vital, a key step in the erectile process is activation of nitric oxide synthase to produce nitric oxide. There are three isoforms of nitric oxide, neuronal constitutive form, endothelial constitutive form, and a cytokine inducible form (Marletta, 1995). A majority of the neurons in the major pelvic ganglion have been shown to contain neuronal nitric oxide synthase (Keast, 1992) and (Ding *et al.*, 1995). In addition, these same neurons contain vasoactive intestinal polypeptide and acetylcholine (Keast, 1992). It appears that the source of most nitric oxide in the penis is neural. Nitric oxide also appears to mediate erectile function by acting in the central nervous system. Application of nitric oxide synthase inhibitors in rat brain areas responsible for erection inhibit the erectile response (Melis *et al.*, 1995). It is also known that dopamine, oxytocin, and serotonin modulate the erectile response and sexual behavior through actions in the paraventricular nucleus and preoptic area of the rat hypothalamus (Melis *et al.*, 1995; Sachs, 1995). However, any connection between nitric oxide and these classical transmitters is yet to be

determined.

Nitric oxide synthase catalyzes the conversion of L-arginine to L-citrulline and nitric oxide. This is a calcium/calmodulin dependent pathway (Marletta, 1995). The mechanism by which this enzyme is activated is unclear. It has been demonstrated in dispersed smooth muscle cells that vasoactive intestinal polypeptide activates nitric oxide synthase by binding a receptor-bound G protein (Murthy *et al.*, 1994). However, the activation process has not been described in the corpus cavernosum. Nitric oxide synthase activity has been shown to be androgen dependent in rat corpus cavernosum (Penson *et al.*, 1996). In addition, aged rats show decreased nitric oxide synthase activity leading to erectile dysfunction (Garban *et al.*, 1995).

Vasoactive intestinal polypeptide is a key neurotransmitter implicated in activation of nitric oxide in the penis, although the precise role is unclear. Vasoactive intestinal polypeptide belongs to a family of peptides from the same precursor molecule. This family includes peptide histidine methionine and pituitary adenylate cyclase activating polypeptide (Kirkeby *et al.*, 1992; Absood *et al.* 1992). Each of these related peptides has relaxant effects on cavernous tissue. Addition of vasoactive intestinal polypeptide to human (Kirkeby *et al.*, 1992) and rabbit (Kim *et al.*, 1995) corpus cavernosum causes relaxation in a dose dependent manner *in vitro*. However, *in vivo* studies have shown that vasoactive intestinal polypeptide causes only partial erection in the rat (Suh *et al.*, 1995) and dog (Yeh *et al.*, 1994). The function of this neurotransmitter and its related peptides have yet to be determined in the corpus cavernosum.

Vasoactive intestinal polypeptide is known to increase cAMP levels in vascular smooth muscle (Absood *et al.*, 1992) and cavernosum smooth

muscle (Hedlund *et al.*, 1995). However, increases in cAMP have not been related to the erectile process. This casts doubt on a primary role for this transmitter in erection. In addition, the use of nitric oxide synthase inhibitors did not affect vasoactive intestinal polypeptide induced relaxations in human corpus cavernosum *in vitro* while they significantly inhibited neurogenic relaxation (Pickard *et al.*, 1993). Also, in tissue made unresponsive to vasoactive intestinal polypeptide by repeated exposure, the relaxation response of cavernous smooth muscle to nerve stimulation did not differ from control (Hayashida *et al.*, 1996). It appears that although vasoactive intestinal polypeptide has some effect in the process of tumescence, its role is independent of the nitric oxide activation step in the primary pathway. Vasoactive intestinal polypeptide, along with acetylcholine, has accessory roles in erection to the primary action of nitric oxide.

Nitric oxide and vasoactive intestinal polypeptide do appear to interact in smooth muscle. As mentioned earlier, these two neurotransmitters are coexpressed in the nerve terminals of neurons in the major pelvic ganglion innervating the penis. Stimulation of the vagus nerve stimulates release of both nitric oxide and vasoactive intestinal polypeptide (Takahashi & Owyang, 1995). In addition, in non-adrenergic non-cholinergic neurons regulating gastric smooth muscle relaxation nitric oxide enhances the release of vasoactive intestinal polypeptide (Grider *et al.*, 1992). Any interaction between these two neurotransmitters in corpus cavernosum has yet to be demonstrated. However, in dispersed and gastric smooth muscle cells, vasoactive intestinal polypeptide stimulates nitric oxide production. Therefore, it appears that direct nitric oxide release from non-adrenergic non-cholinergic neurons is the primary effector in the erectile response pathway.

Nitric oxide has been clearly demonstrated as the key neurotransmitter in stimulation of tumescence. Nitric oxide synthase inhibitors reduce the relaxation response in cavernosum smooth muscle in response to electrical field stimulation (Rajfer *et al.*, 1992). In addition, nitric oxide donors, such as sodium nitroprusside, nitroglycerine, and 3-morpholinosydnonimin, have been shown to relax cavernous tissue *in vitro* in the rat (Argiolas, 1994) and rabbit (Holmquist *et al.*, 1993). The method by which nitric oxide relaxes smooth muscle has been well outlined. Nitric oxide application causes a direct increase in cGMP levels in the corpus cavernosum (Holmquist *et al.*, 1993). Cyclic-GMP levels have been shown to mediate smooth muscle relaxation in this tissue. Addition of cGMP phosphodiesterase inhibitors enhances the relaxation response to electrical field stimulation and nitric oxide donors (Rajfer *et al.*, 1992). The oral drug sildenafil, a potent inhibitor of cGMP phosphodiesterase type V, acts dose dependently to relax rabbit and human cavernous tissue (Stief *et al.*, 1998). Furthermore, in the rabbit, sildenafil has been shown to dose dependently increase cavernous cGMP levels while not effecting cAMP levels (Jeremy *et al.*, 1997). In addition to phosphodiesterase inhibitors, methylene blue, a cGMP formation antagonist, inhibits the smooth muscle relaxation response to nitric oxide (Kim *et al.*, 1991; Azadzoi *et al.*, 1992; Pickard *et al.*, 1993). It is clear that nitric oxide activates guanylate cyclase to form cGMP in the corpus cavernosum. This is the primary event in the process leading to cavernous smooth muscle relaxation and tumescence.

Nitric oxide was demonstrated to activate soluble guanylate cyclase in several smooth muscle types (Arnold *et al.*, 1977). Guanylate cyclase is a hemoprotein, and nitric oxide binds to the heme portion to activate the enzyme (Marletta, 1995). There are two general classes of guanylate

cyclases, soluble and membrane bound (review in Vaandrager & de Jonge, 1996). The soluble form of the enzyme is a heterodimer of α and β subunits. Isoforms of each subunit exist, but are as of yet unidentified. There are six known isoforms of the membrane bound guanylate cyclase, which respond in most cases to natriuretic peptides (review in Vaandrager & de Jonge, 1996). The activation of soluble guanylate cyclase by nitric oxide results in increased intracellular levels of cGMP in smooth muscle cells. Guanylate cyclase inhibitors reverse the relaxant action of nitric oxide in vascular smooth muscle (Olson *et al.*, 1997). The increase in cGMP levels leads directly to smooth muscle cell relaxation. Addition of 8-Br-cGMP, a cell membrane soluble analogue of cGMP, causes cavernosum (Trigo-Rocha *et al.*, 1993b) and penile artery (Simonsen *et al.*, 1995) relaxation in the dog and horse respectively. Cyclic GMP causes corpus cavernosum smooth muscle to relax by binding a variety of intracellular proteins.

Cyclic GMP primarily binds to three types of intracellular proteins in order to elicit smooth muscle relaxation: cGMP-dependent protein kinase, cGMP-regulated ion channels, and cGMP-regulated phosphodiesterases (Lincoln & Cornwell, 1993). The effects of activating cGMP-regulated phosphodiesterases lead to cavernous relaxation by interfering with cAMP-mediated muscle tone. Activation of cGMP-dependent protein kinase and cGMP-regulated ion channels functions to increase calcium efflux while inhibiting influx (review in Vaandrager & de Jonge, 1996). Of these effects, those associated with cGMP-dependent protein kinase, or protein kinase G, appear to be paramount in the corpus cavernosum. Two isoforms of protein kinase G have been isolated in man (Vaandrager & de Jonge, 1996). Type I has been localized as the predominant isoform in vascular tissue. Two isoforms of type I protein kinase exist, α isoform, appearing in lung and

heart tissue, and the β isoform, which is mainly in vascular smooth muscle (review in Vaandrager & de Jonge, 1996). Type II protein kinase G is tightly bound to the plasma membrane, whereas type I is commonly a cytosolic protein (review in Vaandrager & de Jonge, 1996). Type I protein kinase G of the β isoform appears to be the primary kinase involved in penile erection.

The role of protein kinase G has been made clear in vascular tissue relaxation. Addition of (Rp)-8-bromo-PET-cyclic-GMP, an inhibitor of protein kinase G through preventing cGMP activation, to human (Butt *et al.*, 1995) and rabbit (Nakazawa & Imai, 1994) vascular smooth muscle type I protein kinase G inhibits the relaxation process in response to nitric oxide donors. It appears that activation of this kinase is vital to the nitric oxide induced relaxation of smooth muscle. Protein kinase G regulates smooth muscle relaxation by phosphorylating a variety of proteins responsible for regulating cellular calcium levels. One well described pathway of protein kinase G action is through phosphorylation of calcium-activated potassium channels (Yamakage *et al.*, 1996). These channels, which result in calcium influx, are stimulated by nitric oxide activation of cGMP and subsequent kinase activity in smooth muscle. This channel phosphorylation results in cell hyperpolarization, thus inhibiting calcium influx (Yamakage *et al.*, 1996). In addition, protein kinase G inhibits phospholipase C mediated synthesis of inositol triphosphate (an intracellular calcium mobilizer), activates a calcium ATPase pump, directly inhibits voltage gated calcium channels, stimulates a sodium/calcium exchanger, and inhibits inositol triphosphate receptor activity (review in Vaandrager & de Jonge, 1996). All of these effects lead to a reduction of intracellular calcium levels providing for smooth muscle cell relaxation. Furthermore, protein kinase G has been shown to work in concert with protein kinase A to induce relaxation of

dispersed smooth muscle cells (Murthy & Makhlouf, 1995).

The proposed nitric oxide - cGMP - protein kinase relaxation pathway is not the only hypothesis for cavernosum relaxation. In human corpus cavernosum, nitric oxide was shown to directly activate a sodium-potassium-ATPase, independently of cGMP activation (Gupta *et al.*, 1995). Activation of this ATPase causes smooth muscle relaxation by causing hyperpolarization of the cell, thus preventing calcium entry by closure of voltage gated calcium channels. This alternate pathway for corpus cavernosum relaxation appears to function separately from the cGMP pathway, although each plays a role in the overall development of tumescence.

The erectile process, regardless of activation pathway, has been shown to be androgen dependent in the rat (Mills *et al.*, 1992; Leipheimer & Sachs, 1993; Giuliano *et al.*, 1993; Heaton & Varrin, 1994; Lugg *et al.*, 1995b). In these studies, castration resulted in loss of erectile function. In addition, application of exogenous testosterone restored the relaxation response of the corpus cavernosum in castrate animals to levels reported for intact animals. However, studies in the rabbit did not show a decline in cavernous responsivity in castrate animals (Holmquist *et al.*, 1994). The mechanisms by which testosterone regulates rat cavernous tissue remain unclear. It appears that there may be numerous sites of regulatory activity.

Testosterone is synthesized by Leydig cells in the testes. The hypothalamus and the pars anterior of the pituitary gland regulate this process. The hypothalamus synthesizes luteinizing hormone releasing hormone and releases it into the hypophyseal portal blood. This hormone binds to receptors on gonadotrophs in the pars anterior stimulating synthesis of luteinizing hormone. Luteinizing hormone is then released into the

general circulation and gains access to Leydig cells in the testes. Upon stimulation of the leydig cells by luteinizing hormone, cholesterol is synthesized from acetate (Eik-Nes, 1970). The Leydig cell then synthesizes the precursor hormone pregnenolone from cholesterol. Pregnenolone then serves as a precursor for both progesterone and testosterone. Through a series of biochemical reactions the Leydig cells then produce testosterone (review in Eik-Nes, 1970).

Once synthesized, testosterone is secreted into the general circulation. The hormone exerts powerful negative feedback action on both the gonadotrophs of the pars anterior and the hypothalamus. This process regulates serum testosterone levels. Several enzymes may also modify testosterone. One such alteration is the conversion of testosterone to estradiol via aromatase. Through conversion to estradiol, testosterone exerts many central nervous system effects (review in Eik-Nes, 1970). In addition, testosterone is converted by 5-α-reductase into dihydrotestosterone. Dihydrotestosterone has been strongly implicated as the active form of testosterone in regulating erectile function. Application of finasteride, a 5-α-reductase inhibitor, to castrate animals with testosterone replacement, greatly inhibited the erectile response to electric field stimulation (Lugg *et al.*, 1995b). Whereas, dihydrotestosterone did restore erectile function to intact control levels. Regardless of the active testosterone form, the erectile process is clearly androgen dependent.

The striated muscles and vascular smooth muscles involved in erection have been demonstrated to be androgen dependent (Leipheimer & Sachs, 1993). Excision of the striated muscles combined with castration resulted in a decline in the intensity of erections. Testosterone application restored certain responses to intact levels indicating that the vascular

mechanisms that regulate erection are androgen sensitive. Testosterone has also been shown to act directly in the corpus cavernosum. The rate of venous blood outflow is greater in castrate than in intact animals (Mills *et al.*, 1994). This may account for the loss of erectile function in castrate groups and may be a key site of androgen action. These two physical processes mediated by testosterone are not alone as potential regulatory sites. Nitric oxide synthase activity has been shown to be androgen dependent (Lugg *et al.*, 1995b). Castration resulted in a decrease in nitric oxide activity and a corresponding loss of erectile function. Nitric oxide synthase is the vital enzyme for nitric oxide production in the penis. It appears that testosterone may regulate this enzyme by binding to postganglionic neurons containing nitric oxide synthase (Giuliano *et al.*, 1993). In addition, it now looks like testosterone regulates steps downstream of nitric oxide synthase in the nitric oxide - cGMP - protein kinase G pathway. Application of testosterone to aged rats restores erectile function without affecting nitric oxide synthase activity (Garban *et al.*, 1995). In addition, preliminary work done in this lab has shown that the response to nitric oxide addition to cavernosum tissue *in vitro* is androgen dependent (Leipheimer & Toepfer, 1996). Cavernous smooth muscle strips isolated from castrated animals exposed directly to nitric oxide relaxed less than controls, while testosterone application restored the response to intact levels. These results suggest sites of testosterone action distal to nitric oxide synthase regulation or striated muscle control.

Further evidence of a cellular role of testosterone arises from work done with androgen receptors in the penis. Androgen receptor levels are normally low in adult cavernosum tissue (Rajfer *et al.*, 1980). The decline of androgen receptors from youth into adulthood coincides with the cessation of penile growth in the rat (Mills *et al.*, 1996). However, high levels of

testosterone have been shown to upregulate androgen receptor levels (Lin *et al.*, 1993). In addition, application of the androgen receptor inhibitor flutamide greatly reduced the erectile response to electric field stimulation (Penson *et al.*, 1996). Furthermore, castrate animals showed far greater inhibition of tumescence than intact animals. This study demonstrated that testosterone regulates cellular processes as well as other steps in the relaxation pathway leading to erection.

The present study was designed to investigate the effects of castration on rat corpus cavernosum upon addition of the nitric oxide donor, sodium nitroprusside. In addition, application of testosterone to castrate animals was tested to confirm that testosterone restores smooth muscle function. Animals were divided into three treatment groups: intact, castrate, and castrate plus testosterone. Responses of strips of corpus cavernosum to the addition of sodium nitroprusside in each group were compared.

Second, we investigated testosterone's role in regulating guanylate cyclase. The addition of 8-Br-cGMP bypassed the actions of endogenous guanylate cyclase in the nitric oxide - cGMP - protein kinase G pathway. Again, animals were divided into treatment groups as explained above and responses to 8-Br-cGMP were compared. These data were used to characterize the role of testosterone in the corpus cavernosum.

Materials and Methods

Animals

Thirty-three mature male Long/Evans rats (average age 6 months) were used in these experiments. The intact (control) group consisted of 12 males treated with sham castrate surgery via a midline scrotal incision. The tunica albuginea surrounding the testes was also cut, however the testes were not removed. The incisions were then closed with 3-O silk suture. All animals were anesthetized with ketamine, 50 mg / kg, and rompun, 8 mg / kg, injected intramuscularly. The castrate group consisted of 12 males with testes and epididymides removed through similar incisions as described above. The testosterone replacement group consisted of 9 castrated males which were implanted subcutaneously with testosterone containing capsules, SILASTIC medical grade tubing, Dow Corning, Midland, Michigan. The capsules were 40 mm in active length and 1.6 mm ID, 3.2 mm OD. The tubing was sealed on each end with a 5 mm length portion of a wooden applicator and SILASTIC medical adhesive silicon type-A, Dow Corning, Midland, Michigan. These capsules were constructed with modification from the methods described by (Smith *et al.*, 1977). and are similar to capsules previously reported to maintain reflexive erections in ex copula tests (Leipheimer & Sachs, 1993). All animal groups were allowed at least 3 weeks recovery time prior to experimental use.

Tissue Specimens

On the day of the experiment, rats were quickly euthanized with CO_2 and sections of the rat penis, approximately one inch long, were obtained and immediately placed in modified Krebs solution on ice, see composition below. Removal of the surrounding connective tissue, urethra, and dorsal

penile vein was performed under a dissecting microscope to expose the corpus cavernosum tissue. The penis was longitudinally bisected along the central channel into two smooth muscle strips. The isolated tissue sections were individually tied to a glass anchor rod and on the opposite end to a thin connecting wire with silk suture. This tissue assembly was placed in a 40 ml modified Krebs solution bath chamber maintained at 37° C. The modified Krebs bath was continuously bubbled with oxygen (95% O_2, 5% CO_2) to maintain oxygenation and pH. Wet tissue weights were taken immediately after the experiments.

Experimental Design

Once in the tissue bath, the smooth muscle strips were attached by the wire to a hook on a Grass Instruments FT03 Force Displacement Transducer, Quincy, Massachusetts. The transducer signal output was amplified by Grass Instruments model P122 strain gage amplifier. The signal was then received by a Grass Instruments Polyview, version 1.0, 4-channel recorder software program. The transducer was calibrated to measure force in mg. An average resting tension of 175.1 +/- 5.52 mg, 167.6 +/- 5.00 mg, and 173.2 +/- 2.82 mg was then placed on the corpus cavernosum strips from intact, castrate, and testosterone replacement groups respectively, using hand turned tension adjusters. The tissue was then allowed to equilibrate for 1 hour. After this period, the tissue strips from all groups were contracted by adding 10 ml of norepinephrine directly to the tissue bath, resulting in a final concentration of 10^{-4} M. Specificity of the smooth muscle response to norepinephrine was previously demonstrated in this laboratory using the α-adrenergic receptor blocker, phentolamine (Leipheimer & Toepfer, 1996). The tissue contracted for 15-20 minutes until a stable higher tension baseline was established. The force of contraction (mg) was recorded for each corpus

cavernosum strip prior to addition of sodium nitroprusside or 8-bromo-cGMP. Contractions less than 75 mg were not used for further relaxation experiments in order to maximize the relative value of percentage data and to minimize any effect of transducer drift on the data. The transducers were later shown to increase on average 2.83 +/- 2.56 mg over the experimental time course of 90 minutes.

Experiment I : Measurement of SNP Induced Relaxation

One ml of SNP, resulting in a final concentration of 10^{-4} M, was added to the norepinephrine contracted tissue strips isolated from intact, castrate, and testosterone replacement animals. The corpus cavernosum strips were allowed 25-30 minutes to achieve maximum relaxation. Next, 1 ml of sodium nitroprusside, resulting in a final concentration of 10^{-3} M, was cumulatively added to the chambers. Again, 25-30 minutes were allowed to record the decrease in tension due to relaxation. At the end of this period tension values in mg were converted to percent relaxations of the total contraction.

Experiment II : Measurement of 8-bromo-cGMP Induced Relaxation

In this experiment rats were divided into treatment groups as described in experiment I. One ml of 8-bromo-cGMP, resulting in a final concentration of 10^{-5} M, was added to each norepinephrine-contracted tissue strip. These corpus cavernosum strips were recorded for 25-30 minutes to attain maximum relaxation. Next, 1 ml of 8-bromo-cGMP, resulting in a final concentration of 10^{-4} M, was added to the chamber. Once again, 25-30 minutes were allowed to record maximum relaxation. The mg tension data was then converted to percent relaxation of the peak norepinephrine-induced contraction.

Drugs and Solutions

The modified Krebs solution used was of the following concentrations: NaCl (119 mM); KCl (4.6 mM); $NaHCO_3$ (15 mM); $CaCl_2$ (1.5 mM); MgCl (1.2 mM); NaH_2PO_4 (1.2 mM); and glucose (11 mM) (Hedlund *et al.*, 1995). This solution was then adjusted to pH 7.2-7.4. Norepinephrine bitartrate salt, sodium nitroprusside dihydrate, 8-bromo-cGMP, and testosterone were obtained from Sigma, St. Louis, Missouri. Solutions of norepinephrine, sodium nitroprusside, and 8-bromo-cGMP were made in modified Krebs solution.

Statistical Analysis

Percentage data acquired was analyzed, after Arcsin transformation, using the SigmaStats program, version 1.0, Jandel Scientific, Corte Madera, California. Data was subjected to a one-way ANOVA, with $p < 0.05$, to determine significance. Differences between treatment groups were probed using the Student-Newman-Keuls multiple comparison test, with $p < 0.05$ set as the minimum level for significance. SigmaPlot, version 1.02, Jandel Scientific, Corte Madera, California, was then used to construct graphical representations of the percentage data.

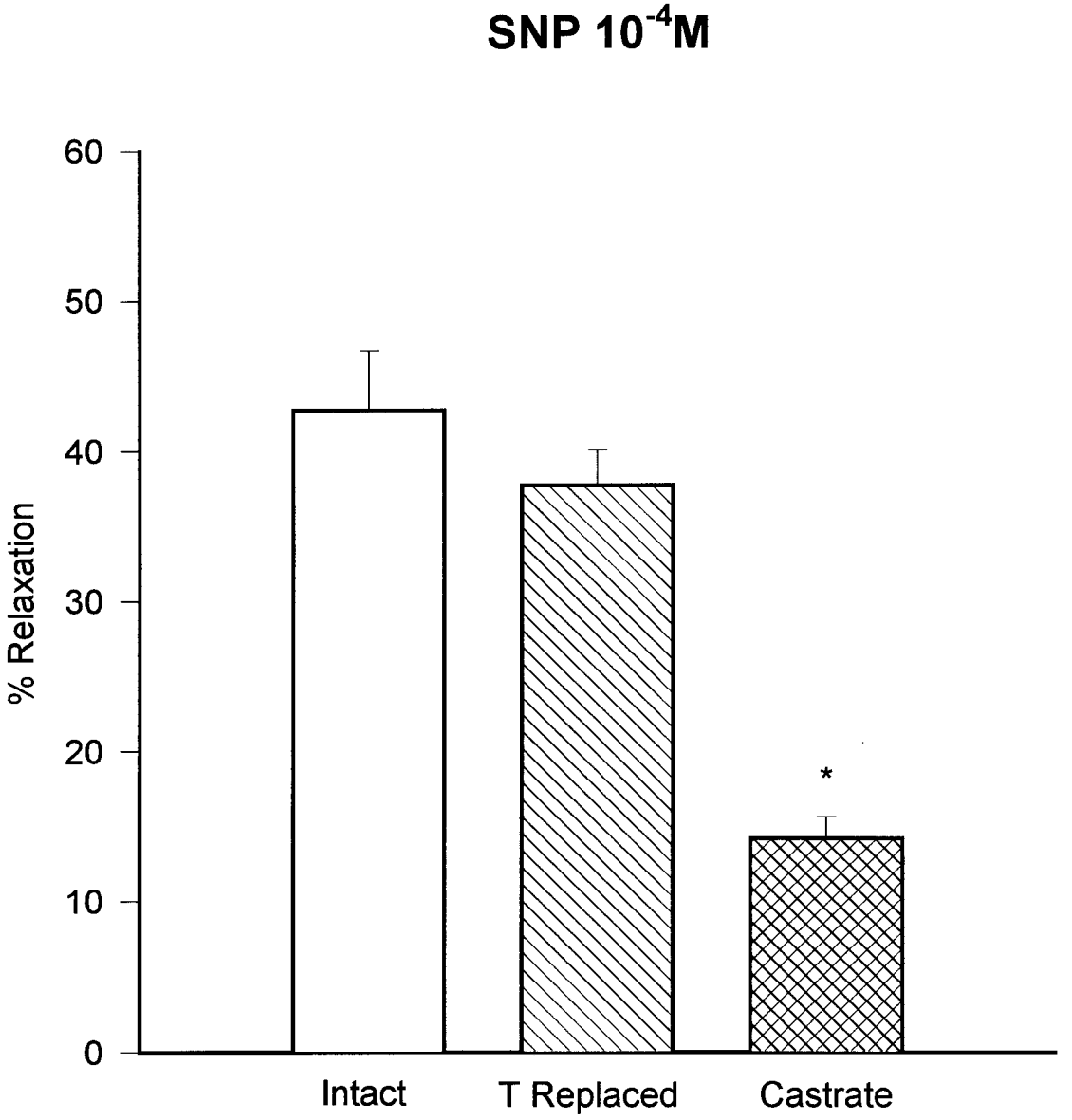
Results

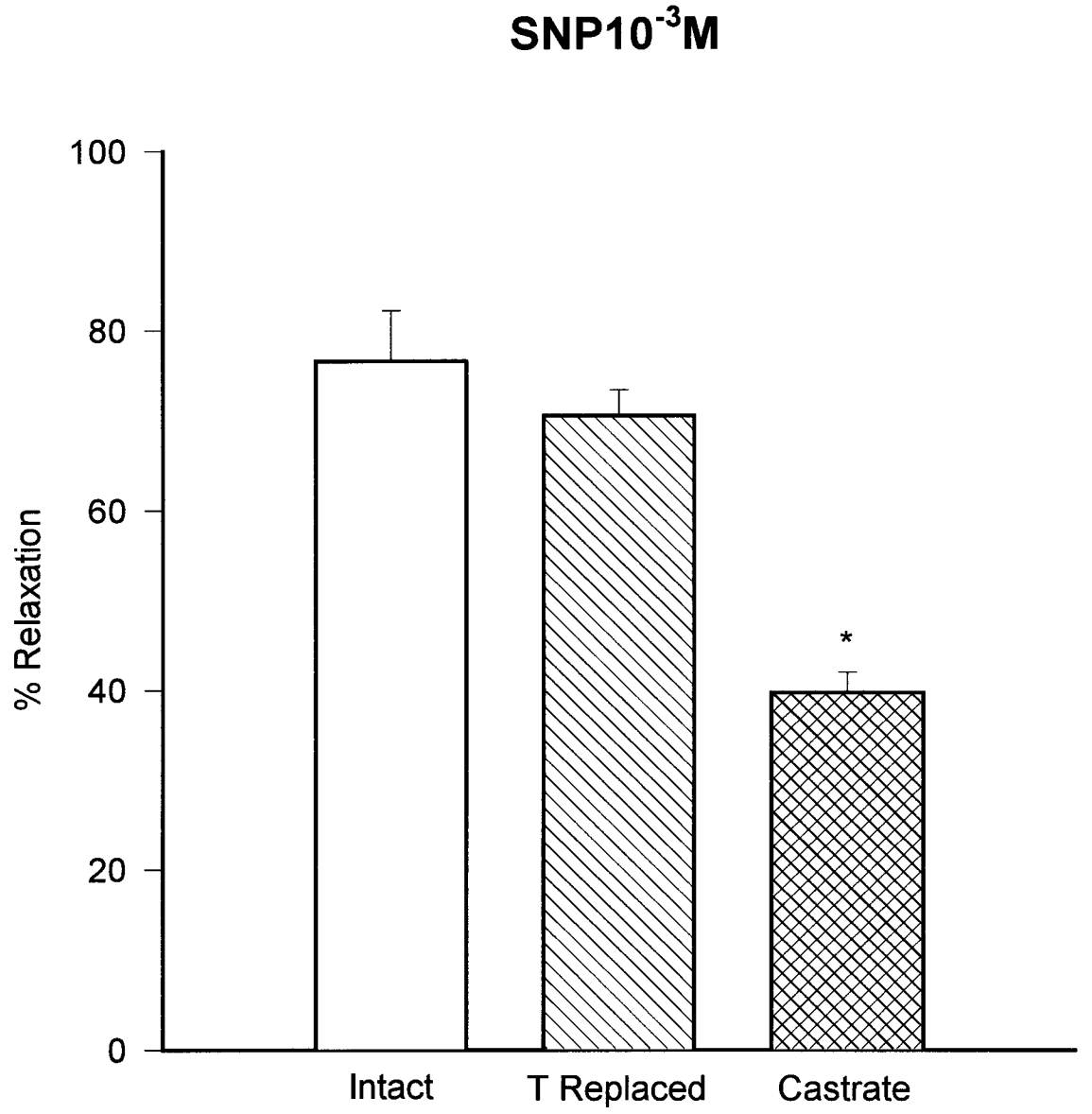
The average tissue weights of isolated corpus cavernosum strips used in these experiments were 62.9 +/- 2.12 mg for intact (control), 62.7 +/- 2.05 mg for castrate, and 80.8 +/- 1.84 mg for testosterone replacement animals. Intact and castrate tissue weights were not significantly different, while the weight of the tissue from testosterone replaced rats was significantly higher, $p < 0.001$. The peak norepinephrine (10^{-4} M) stimulated contractions were as follows; 106.2 +/- 6.29 mg in the intact group, 112.9 +/- 10.50 mg for the castrate group, and 130.9 +/- 11.54 mg in the testosterone replacement group. These increases in tension were not significantly different between treatment groups, $p = 0.183$ one-way ANOVA. Furthermore, norepinephrine contractions when expressed as mg tension per mg tissue weight were not significantly different between treatment groups, $p = 0.266$ one-way ANOVA.

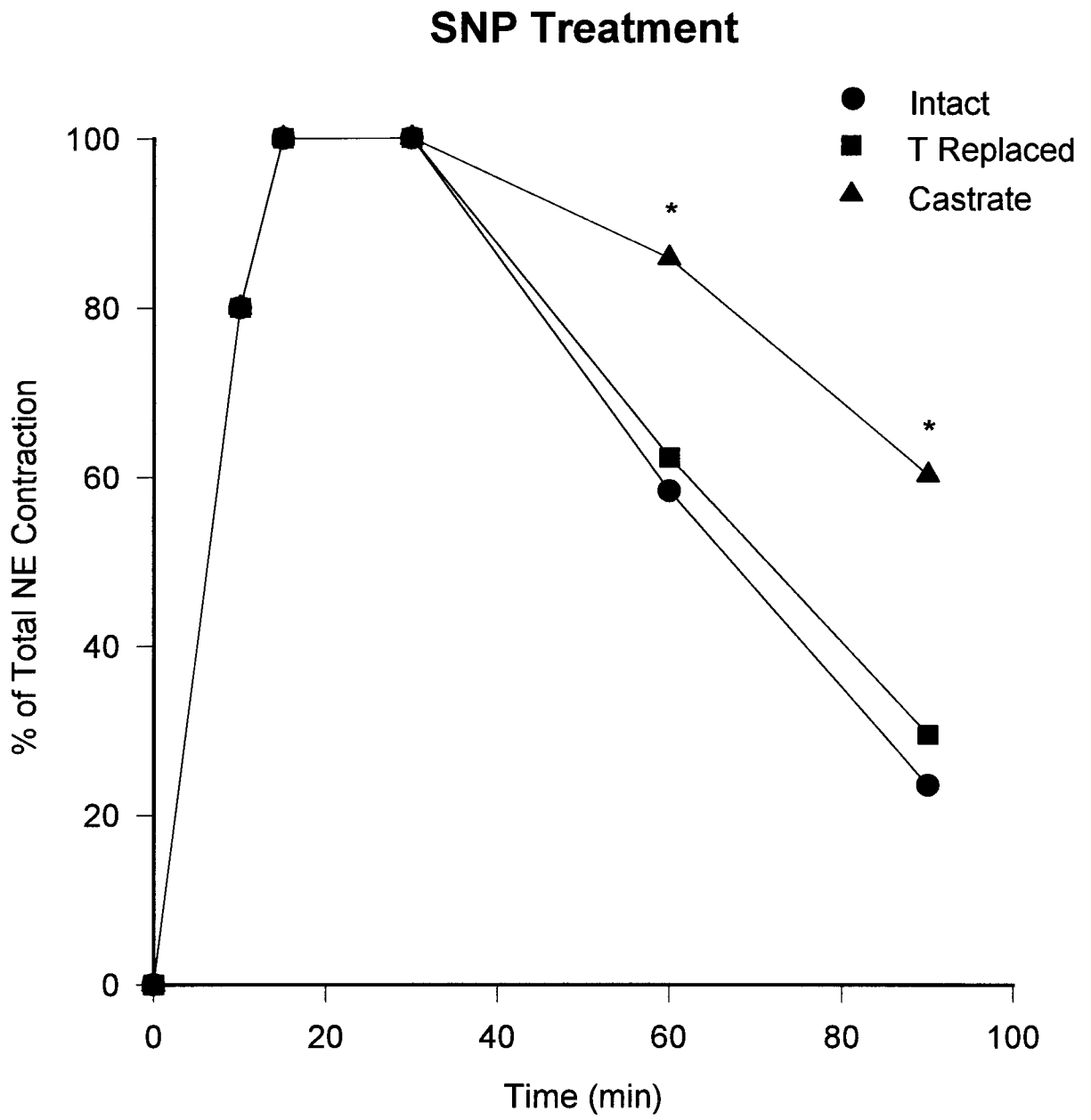
Data collected from experiment I are summarized in figures 1 and 2 for isolated corpus cavernosum strips treated with 10^{-4} M and 10^{-3} M sodium nitroprusside, respectively. Data is presented as the percent relaxation from the peak norepinephrine-induced contraction. In the sodium nitroprusside 10^{-4} M trials, the mean percent relaxations were 42.7 +/- 3.97% ($n = 7$) for the intact group, 37.7 +/- 2.39% ($n = 8$) for the testosterone replacement group, and 14.2 +/- 1.45% ($n = 7$) for the castrate group (fig. 1). Analysis of variance indicated significance between groups, $p < 0.0000008$. Treatment with 10^{-3} M sodium nitroprusside (fig. 2) produced similar results. The mean relaxations were 76.5 +/- 5.69% ($n = 7$) for intact, 70.5 +/- 2.86% ($n = 8$) for testosterone replacement, and 39.8 +/- 2.26% ($n = 7$) for castrated animals. Again, one-way ANOVA revealed significance between groups, $p <$

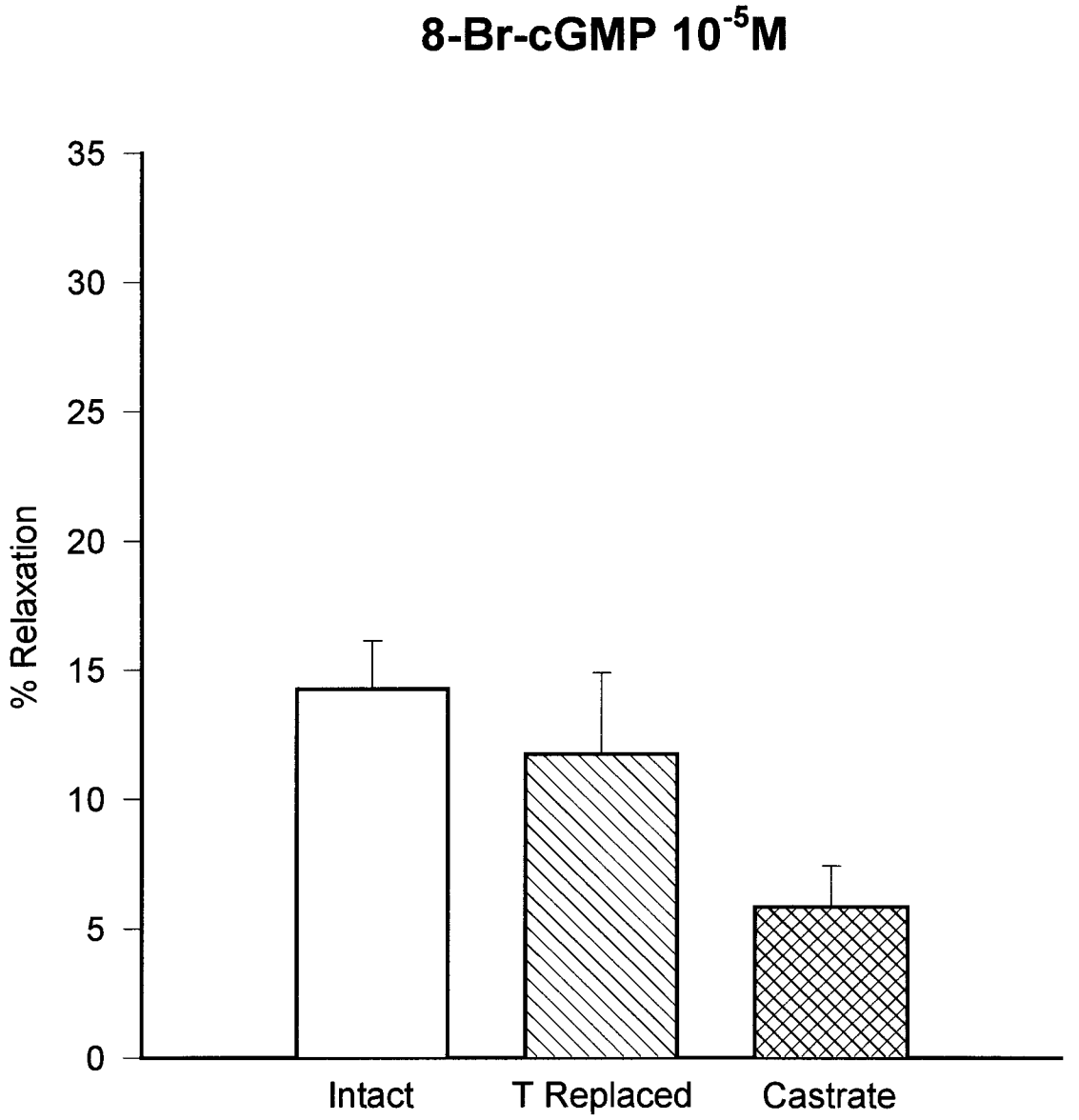
0.00004. In these experiments, castration significantly inhibited the degree of relaxation following treatment with 10^{-4} M ($p < 0.001$) and 10^{-3} M ($p < 0.001$) sodium nitroprusside. A graphical representation of the experimental time course is presented in figure 3. This graph depicts changes in tension over time (expressed as percent of norepinephrine-induced contraction) throughout the entire experiment. Each treatment group is depicted by mean percent relaxation values. Peak norepinephrine-induced tensions are expressed as 100 % while the zero value represents resting tension prior to addition of norepinephrine to the tissue.

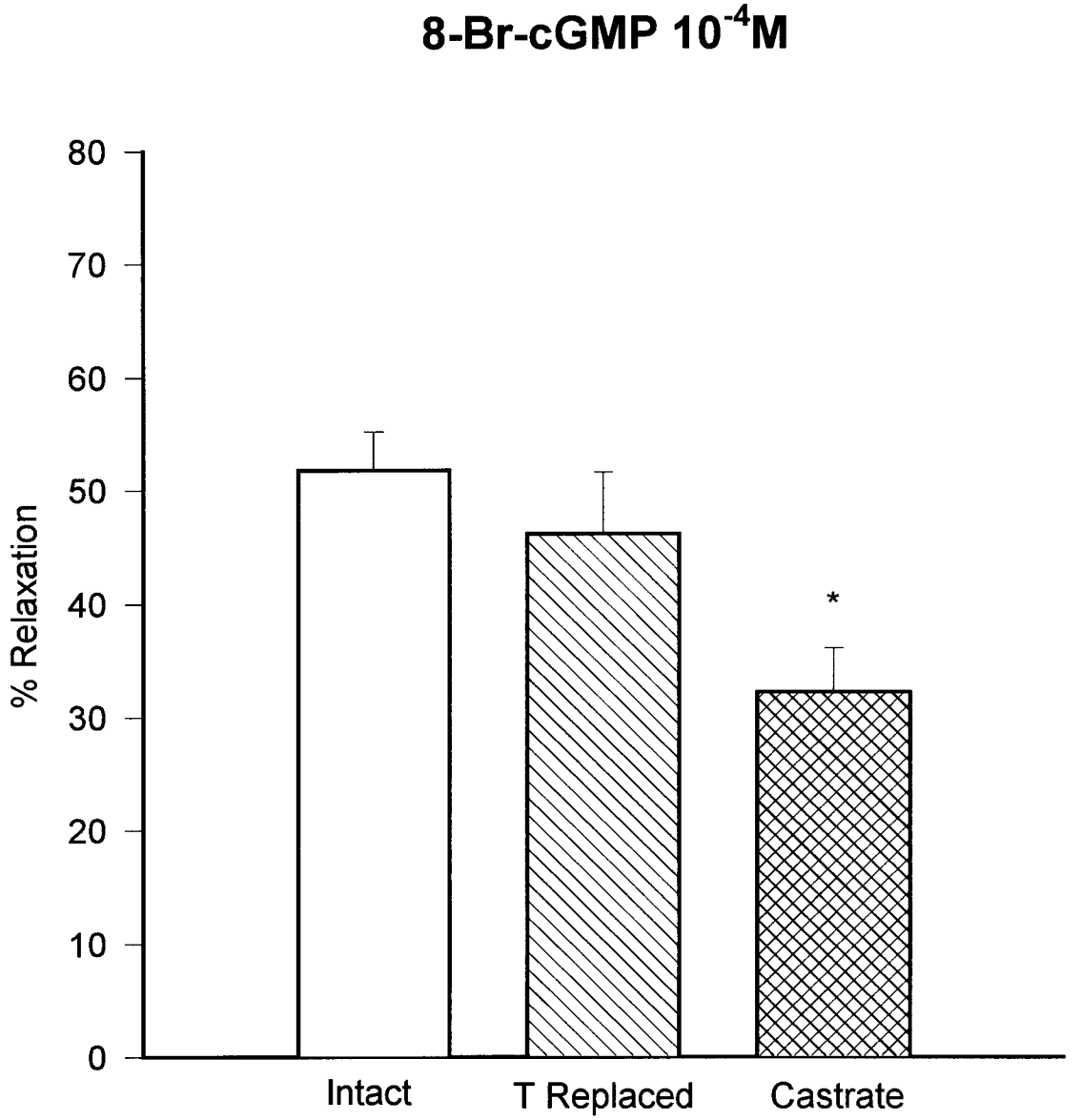
Data collected from experiment II is summarized in figures 4 and 5 for 10^{-5} M and 10^{-4} M doses of 8-bromo-cGMP respectively. Again, relaxation data is treated as percent decrease from the peak norepinephrine-induced contracted level. Corpus cavernosum strips treated with 10^{-5} M 8-bromo-cGMP relaxed as follows; 14.3 +/- 1.85% (n = 8) for intact, 11.7 +/- 3.15% (n = 8) for testosterone replaced, and 5.8 +/- 1.59% (n = 7) for the castrated animals (fig. 4). The differences between these treatment groups were not significant, $p = 0.0689$. In contrast, castration significantly reduced the degree of relaxation after treatment with 10^{-4} M 8-bromo-cGMP (fig. 5), $p < 0.02$, while testosterone treatment restored relaxation values to control levels. The mean percent relaxation values were 51.8 +/- 3.43% (n = 8) for intact, 46.2 +/- 5.43% (n = 8) for testosterone replacement, and 32.2 +/- 3.89% (n = 7) for castrated rats. Similar to experiment I, intact and testosterone replaced groups were not different from each other, while the castrate group was different from each, $p < 0.025$ versus intact and $p < 0.05$ versus testosterone replaced. A graphical representation of the 8-bromo-cGMP experiment is depicted in figure 6. Changes in tension are expressed in the same manner as in figure 3.

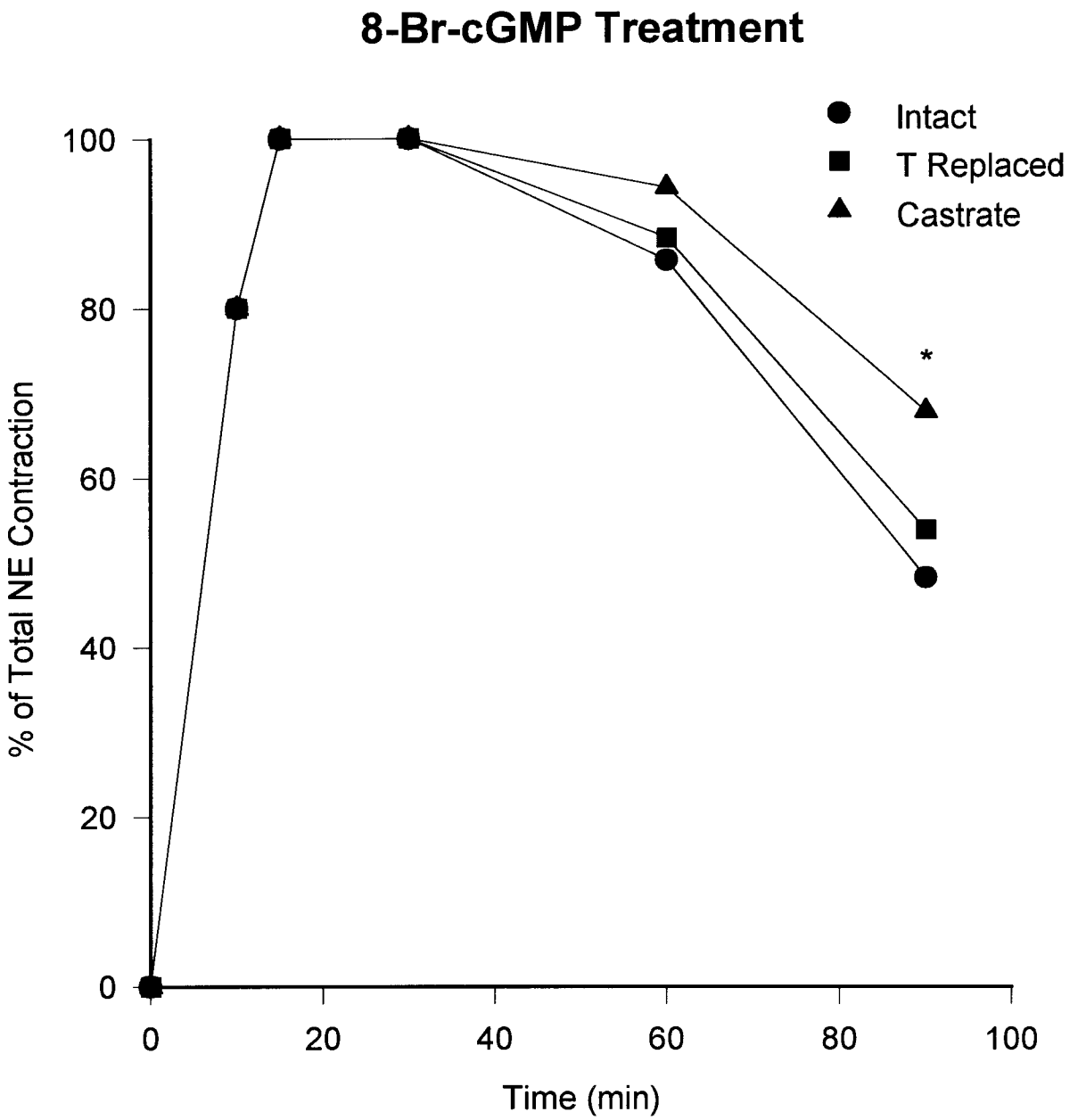

SNP 10-4M
60
50
40
30
20
10
0
% Relaxation
*
Intact
T Replaced
Castrate


SNP10-3M
100
80
60
40
20
0
% Relaxation
*
Intact
T Replaced
Castrate


SNP Treatment
Intact
T Replaced
Castrate
100
80
60
40
20
0
% of Total NE Contraction
0
20
40
60
80
100
Time (min)
*
*


8-Br-cGMP 10-5M
% Relaxation
35
30
25
20
15
10
5
0
Intact
T Replaced
Castrate


8-Br-cGMP 10-4M
% Relaxation
80
70
60
50
40
30
20
10
0
*
Intact
T Replaced
Castrate


8-Br-cGMP Treatment
Intact
T Replaced
Castrate
*
100
80
60
40
20
0
% of Total NE Contraction
0
20
40
60
80
100
Time (min)

Discussion

This study clearly demonstrated that castration inhibits the relaxation of isolated corpus cavernosum following treatment with either sodium nitroprusside or 8-bromo-cGMP. In addition, testosterone replacement in castrated rats was able to restore relaxation to control levels. These results suggest that androgens act in corpus cavernosum smooth muscle to regulate erectile function via the proposed nitric oxide-guanylate cyclase-cGMP pathway (Lue & Tanagho, 1987). Our results also indicate that the loss, in castrates, of the ability to respond to relaxing agents such as sodium nitroprusside is not due to tissue mass differences, but is due to androgen regulation. In this study, there was no significant atrophy of the corpus cavernosum in castrated rats. Furthermore, there were no difference in peak tension developed after norepinephrine treatment in the raw data as well as when the data were expressed as mg tension per mg tissue weight. This indicates that the differences in response to relaxing agents between treatment groups were due to differences in androgen levels. The sensitivity of erectile function to androgens has been clearly demonstrated in the rat model (Mills *et al.*, 1992; Giuliano *et al.*, 1993; Heaton & Varrin, 1994; Lugg *et al.*, 1995b; Leipheimer & Sachs, 1993; Mills *et al.*, 1994; Leipheimer & Toepfer, 1996). Although, in the rabbit the role of androgens is less clear (Holmquist *et al.*, 1994). Lugg *et al.* (1995b) demonstrated *in vivo* in the rat that androgen levels control the smooth muscle responsiveness to electrical field stimulation. However, the response of corpus cavernosum to norepinephrine does not appear to be androgen dependent. All treatment groups were contracted similarly by norepinephrine application. Castrated animals showed no change in responsiveness to norepinephrine and

therefore, presumably, no change in the general mechanism of smooth muscle tone. Norepinephrine is the typical neurotransmitter of noradrenergic nerve fibers that innervate the penis to maintain detumescence (Ignarro, 1992).

Results of the present study show that testosterone acts within the smooth muscle cells of the corpus cavernosum to modulate relaxation of this tissue. Addition of sodium nitroprusside (a nitric oxide donor) to the smooth muscle bath resulted in comparable relaxation of corpus cavernosum strips isolated from intact and testosterone replaced rats. In contrast, the relaxation response of tissue from castrated animals was greatly inhibited. This indicates that testosterone must be acting through modulation of some intracellular step in the nitric oxide-guanylate cyclase-cGMP pathway to influence relaxation of smooth muscle. Lugg *et al.* (1995b) has reported that androgens regulate erectile function by affecting the activity of nitric oxide synthase in neuronal terminals. Although testosterone may regulate nitric oxide synthase levels, we have demonstrated that the addition of sodium nitroprusside directly to smooth muscle, which bypasses the nitric oxide synthase action, is less effective in relaxing tissue from castrated rats. It is now clear that testosterone has a direct action on the responsiveness of corpus cavernosum tissue to nitric oxide *in vitro*. This agrees with studies by Garban *et al.* (1995) which demonstrated that testosterone administration restores erectile function without affecting neuronal nitric oxide synthase levels in aged rats. However, the precise mechanism by which testosterone regulates corpus cavernosum smooth muscle cell relaxation remains unclear. Testosterone in high concentrations has been shown to up regulate androgen receptors in corpus cavernosum smooth muscle (Lin *et al.*, 1993). Although androgen receptor levels are very low in the penile smooth muscle of mature

rats (Rajfer *et al.*, 1980), blockade of androgen receptors in this tissue was reported to obliterate the erectile response in castrates and inhibit the response in intact animals to electric field stimulation with no further reduction of nitric oxide synthase activity (Penson *et al.*, 1996). Whether changes in androgen receptor levels or affinity play a role in testosterone actions remains to be determined. However, it appears that androgen receptor binding is necessary to maintain erectile function. Presumably, androgens bind to their receptor and via classical genomic actions regulate synthesis of intracellular proteins involved in the nitric oxide-guanylate cyclase-cGMP pathway of corpus cavernosum relaxation.

A number of proteins could potentially play key roles in the intracellular events leading to relaxation in this tissue and each could be regulated by androgens. One key intracellular protein that could be a potential androgen target is guanylate cyclase. Soluble guanylate cyclase is activated by nitric oxide and is responsible for converting GTP to cGMP. In the present study 8-bromo-cGMP was added directly to the smooth muscle cell bath to test whether this step is a key site of androgen regulation in the tissue. If androgens act in this tissue to regulate levels or activity of guanylate cyclase then we would expect that addition of 8-bromo-cGMP would result in comparable relaxation among treatment groups. However, the addition of 8-bromo-cGMP did not restore smooth muscle relaxation in tissue isolated from castrated animals to control levels. Although these results do not rule out some degree of androgen regulation of guanylate cyclase levels or activity, guanylate cyclase is probably not the primary site of testosterone regulation in this tissue.

In conclusion, our results clearly demonstrate that testosterone acts intracellularly in corpus cavernosum smooth muscle cells to regulate

relaxation. Testosterone must be acting distal to the site of nitric oxide synthase regulation in the nitric oxide-guanylate cyclase-cGMP pathway. Furthermore, 8-bromo-cGMP did not restore erectile function to castrate animals. This suggests that testosterone must be acting distal to the guanylate cyclase activation step in the pathway. Whether testosterone acts on one major enzyme or on a number of proteins that when activated together result in full erectile function remains to be determined.

References

Absood A, Chen D, Wang Y, Wang Z, Hakanson R. Vascular effects of pituitary adenylate cyclase activating peptide: a comparison with vasoactive intestinal peptide. Regulatory Peptides 1992; 40:323-329.

Andersson KE, Holmquist F. Regulation of tone in penile cavernous smooth muscle. World Journal of Urology 1994; 12:249-261.

Argiolas A. Nitric oxide is a central mediator of penile erection. Neuropharmacology 1994; 33:1339-1344.

Arnold WP, Mittal CK, Katsuki S, Murad F. Nitric oxide activates guanylate cyclase and increases guanosine 3':5'- cyclic monophosphate levels in various tissue preparations. Proc National Acad of Sciences 1977; 74:3203-3207.

Azadzoi KM, Kim N, Brown ML, Goldstein I, Cohen RA, Saenz de Tejada I. Enothelium-derived nitric oxide and cyclooxygenase products modulate corpus cavernosum smooth muscle tone. Journal of Urology 1992; 147:220-225.

Brock G, Hsu GL, Nunes L, Von Heyden B, Lue TF. The anatomy of the tunica albuginea in the normal penis and peyronie's disease. Journal of Urology 1997; 157:276-281.

Burnett AL. Role of nitric oxide in the physiology of erection. Biol. of Reprod. 1995; 52:485-489.

Butt E, Pohler D, Genieser HG, Huggins JP, Bucher B. Inhibition of cyclic GMP-dependent protein kinase-mediated effects by (Rp)-8-bromo-PET-cyclic GMPS. Brit. Journal of Pharm. 1995; 116:3110-3116.

Dail WG, McGuffee L, Minorsky N, Little S. Responses of smooth muscle strips from erectile tissue to drugs and transmural nerve stimulation. Journal Auton. Pharmac. 1987; 7:287-293.

De Groat WC. Anatomy and physiology of the lower urinary tract. Urologic Clinics of NA 1993; 20:383-401.

De Groat WC, Booth AM. Neural control of penile erection. In: , Nervous control of the urogenital system. 1991:465-522.

De Palma RG. New developments in the diagnosis and treatment of impotence. Western Journal of Medicine 1996; 164:54-61.

Ding YQ, Takada M, Kaneko T, Mizuno N. Colocalization of vasoactive intestinal polypeptide and nitric oxide in penis-innervating neurons in the major pelvic ganglion of the rat. Neuroscience Research 1995; 22:129-131.

Eik-Nes KB. Synthesis and secretion of androstenedione and testosterone. In: Eik-Nes KB (ed.), The androgens of the testis. New York: Marcel Dekker; 1970:1-48.

Garban H, Marquez D, Cai L, Rajfer J, Gonzalez-Cadavid NF. Restoration of normal adult penile erectile response in aged rats by long-term treatment with androgens. Biology of Reprod 1995; 53:1365-1372.

Giuliano F, Rampin O, Schirar A, Jardin A, Rousseau JP. Autonomic control of penile erection: modulation by testosterone in the rat. Journal of Neuroendocrinology 1993; 5:677-683.

Goldstein I, Lue TF, Padma-Nathan H, Rosen RC, Steers WD, Wicker PA. Oral sildenafil in the treatment of erectile dysfunction. NE Journal of Medicine 1998; 338:1397-1404.

Gozes I, Fridkin M. A fatty neuropeptide : potential drug for noninvasive impotence treatment in a rat model. Journal of Clinic. Invest. 1992; 90:810-814.

Grider JR, Murthy KS, Jin J, Jin G, Makhlouf GM. Stimulation of nitric oxide from muscle cells by VIP : prejunctional enhancement of VIP release. Amer. Journal of Phys. 1992; 262:774-778.

Gupta S, Moreland RB, Munarriz R, Daley J, Goldstein I, Saenz de Tejada I. Possible role of Na^+ - K^+ - ATPase in the regulation of human corpus cavernosum smooth muscle contractility by nitric oxide. Brit. Journal of Pharm. 1995; 116:2201-2206.

Hayashida H, Okamura T, Tomoyoshi T, Toda N. Neurogenic nitric oxide mediates relaxation of canine corpus cavernosum. Journal of Urology 1996; 155:1122-1127.

Heaton JPW, Varrin SJ. Effects of castration and exogenous testosterone supplementation in an animal model of penile erection. Journal of Urology 1994; 151:797-800.

Hedlund P, Alm P, Ekstrom P, Fahrenkrug J, Hannibal J, Hedlund H, Larsson B, Andersson KE. Pituitary adenylate cyclase-activating polypeptide, helospectin, and vasoactive intestinal polypeptide in human corpus cavernosum. Brit Journal of Pharm 1995; 116:2258-2266.

Holmquist F, Persson K, Bodker A, Andersson KE. Some pre- and postjunctional effects of castration in rabbit isolated corpus cavernosum and urethra. Journal of Urology 1994; 152: 1011-1016.

Holmquist F, Fridstrand M, Hedlund H, Andersson KE. Actions of 3-morpholinosydnonimin (SIN-1) on rabbit isolated penile erectile tissue. Journal of Urology 1993; 150:1310-1315.

Ignarro LJ. Nitric oxide as the physiological mediator of penile erection. Journal of NIH Research 1992; 4:59-62.

Jeremy JY, Ballard SA, Naylor AM, Miller MA, Angelini GD. Effects of sildenafil, a type-5 cGMP phosphodiesterase inhibitor, and papaverine on cyclic GMP and cyclic AMP levels in the rabbit corpus cavernosum in vitro. British Journal of Urology 1997; 79:958-963.

Keast JR. A possible neural source of nitric oxide in the rat penis. Neuroscience Letters 1992; 143:69-73.

Kim N, Azadzoi KM, Goldstein I, Saenz de Tejada I. A nitric oxide-like factor mediates nonadrenergic-noncholinergic relaxation of penile corpus cavernosum smooth muscle. Journal of Clinic. Invest. 1991; 88:112-118.

Kim YC, Kim JH, Davies MG, Hagen PO, Carson CC. Modulation of vasoactive intestinal polypeptide-mediated relaxation by nitric oxide and prostanoids in the rabbit corpus cavernosum. Journal of Urology 1995; 153:807-810.

Kim YC, Davies MG, Hagen PO, Carson CC. Experimental evidence for endothelium dependent relaxation and neuronal nitric oxide in corpus cavernosum. Yonsei Medical Journal 1994; 35:308-313.

Kirkeby HJ, Fahrenkrug J, Holmquist F, Ottesen B. Vasoactive intestinal polypeptide and peptide histidine methionine in human penile corpus cavernosum tissue and circumflex veins : localization and *in vitro* effects. Euro. Journal of Clinic. Invest. 1992; 22:24-30.

Leipheimer RE, Toepfer JR. The effects of castration on nitric oxide-induced relaxation of isolated corpus cavernosum in the rat. In: Program of the Conference on Reproductive Behavior; 1996; Montreal, QE. Abstract 44.

Leipheimer RE, Sachs BD. Relative androgen sensitivity of the vascular and striated-muscle systems regulating penile erection in rats. Phys & Behav 1993; 54:1085-1090.

Lin MC, Rajfer J, Swerdloff RS, Gonzalez-Cadavid NF. Testosterone down-regulates the levels of androgen receptor mRNA in smooth muscle cells from the rat corpora cavernosa via aromatization to estrogens. Journal Steroid Biochem. Molec. Biol. 1993; 45:333-343.

Lincoln TM, Cornwell TL. Intracellular cyclic GMP receptor proteins. FASEB 1993; 7:328-338.

Liu SF, Crawley DE, Evans TW, Barnes PJ. Endothelium-dependent nonadrenergic, noncholinergic neural relaxation in guinea pig pulmonary artery. Journal of Pharm. and Exper. Therapeutics 1992; 260:541-548.

Lue TF, Tanagho EA. Physiology of erection and pharmacological management of impotence. Journal of Urology 1987; 137:829-836.

Lugg JA, Gonzalez-Cadavid NF, Rajfer J. The role of nitric oxide in erectile function. Journal of Andrology 1995a; 16:2-4.

Lugg JA, Rajfer J, Gonzalez-Cadavid NF. Dihydrotestosterone is the active androgen in the maintenance of nitric oxide-mediated penile erection in the rat. Endocrinology 1995b; 136:1495-1501.

Marletta MA. Mechanisms of signal transduction with nitric oxide : studies on nitric oxide synthase and guanylate cyclase. In: Program of the The Protein Society First European Symposium; 1995; Davos, Switzerland.

Matsuzaka J, Aoki H, Fujioka T, Kubo T, Yasuda N. Mechanisms of veno-occlusion within and outside the canine corpus cavernosum penis using a pressure flow technique and cavernous venography. Intern. Journal of Urology 1996; 3:47-53.

Melis MR, Stancampiano R, Argiolas A. Role of nitric oxide in penile erection and yawning induced by 5-HT1c receptor agonists in male rats. Naunyn-Scmied. Arch. of Pharm. 1995; 351:439-445.

Mills TM, Reilly CM, Lewis RW. Androgens and penile erection: a review. Journal or Andrology 1996; 17:633-638.

Mills TM, Stopper VS, Wiedmeier VT. Effects of castration and androgen replacement on the hemodynamics of penile erection in the rat. Biology of Reprod 1994; 51:234-238.

Mills TM, Wiedmeier VT, Stopper VS. Androgen maintenance of erectile function in the rat penis. Biology of Reprod 1992; 46:342-348.

Murthy KS, Makhlouf GM. Interaction of cA-kinase and cG-kinase in mediating relaxation of dispersed smooth muscle cells. Amer. Journal of Physiol. 1995; 268:171-180.

Murthy KS, Makhlouf GM. Vasoactive intestinal peptide/pituitary adenylate cyclase-activating peptide-dependent activation of membrane bound NO synthase in smooth muscle mediated by pertussis toxin sensitive Gi1-2. Journal of Biol. Chem. 1994; 269:15977-15980.

Nakazawa M, Imai S. Rp-8-Br-guanosine-3':5'-cyclic monophosphorothioate inhibits relaxation elicited by nitroglycerin in rabbit aorta. Euro Journal of Pharm 1994; 253:179-181.

Newman HF, Northup JD. Mechanisms of human penile erection: an overview. Urology 1981; 17:399-408.

Olson LJ, Knych ET, Herzig TC, Drewett JG. Selective guanylyl cyclase inhibitor reverses nitric oxide-induced vasorelaxation. Hypertension 1997; 29:254-261.

Penson DF, Ng C, Cai L, Rajfer J, Gonzalez-Cadavid NF. Androgen and pituitary control of penile nitric oxide synthase and erectile function in the rat. Biol. of Reprod. 1996; 55: 567-574.

Pickard RS, Powell PH, Zar MA. Evidence against vasoactive intestinal polypeptide as the relaxant neurotransmitter in human cavernosal smooth muscle. Brit Journal of Pharm 1993; 108:497-500.

Quinlan DM, Nelson RJ, Partin AW, Mostwin JL, Walsh PC. The rat as a model for the study of penile erection. Journal of Urology 1989; 141:656-661.

Rajfer J, Aronson WJ, Bush PA, Dorey FJ, Ignarro LJ. Nitric oxide as a mediator of the corpus cavernosum in response to nonadrenergic, noncholinergic neurotransmission. NE Journal of Med 1992; 326:90-94.

Rajfer J, Namkung PC, Petra PH. Identification, partial characterization and age-related changes of a cytoplasmic androgen receptor in the rat penis. Journal Steroid Biochem 1980; 13:1489-1492.

Sachs BD. Placing erection in context: the reflexogenic-psychogenic dichotomy reconsidered. Neuroscience & Biobehavioral Reviews 1995; 19:211-224.

Sachs BD. Role of striated penile muscles in penile reflexes, copulation and induction of pregnancy in the rat. Journal of Reprod. Fert. 1982; 66:433-443.

Saenz de Tejada I, Kim N, Lagan I, Krane RJ, Goldstein I. Regulation of adrenergic activity in penile corpus cavernosum. Journal of Urology 1989; 142:1117-1121.

Seftel AD, Viola KA, Kasner SE, Ganz MB. Nitric oxide relaxes rabbit corpus cavernosum smooth muscle via a potassium-conductive pathway. Biochem. and Biophys. Research Comm. 1996; 219:382-387.

Simonsen U, Prieto D, Saenz de Tejada I, Garcia-Sacristan A. Involvement of nitric oxide in non-adrenergic non-cholinergic neurotransmission of horse deep penile arteries : role of charybdotoxin-sensitive K^+ channels. Brit. Journal of Pharm. 1995; 116:2582-2590.

Smith ER, Damassa DA, Davidson JM. Hormone administration: peripheral and intracranial implants. In: Myers RD, ed. Methods in psychobiology. vol. 3. New York: Academic Press; 1977: 259-279.

Stief CG, Uckert S, Becker AJ, Truss MC, Jonas U. The effect of the specific phosphodiesterase (PDE) inhibitors on human and rabbit cavernous tissue in vitro and in vivo. Journal or Urology 1998; 159:1390-1393.

Suh JK, Mun KH, Cho CK, Shin HC, Kim YS, Park TC. Effect of vasoactive intestinal peptide and acetylcholine on penile erection in the rat *in vivo*. Inter. Journal of Impot. Research 1995; 7:111-118.

Takahashi T, Owyang C. Vagal control of nitric oxide and vasoactive intestinal polypeptide release in the regulation of gastric relaxation in rat. Journal of Phys. 1995; 484:481-492.

Takahashi Y, Aboseif SR, Benard F, Stief CG, Lue TF, Tanagho EA. Effect of intracavernous simultaneous injection of acetylcholine and vasoactive intestinal polypeptide on canine penile erection. Journal of Urology 1992; 148:446-448.

Trigo-Rocha F, Aronson A, Hohenfellner M, Ignarro LJ, Rajfer J, Lue TF. Nitric oxide and cGMP: mediators of pelvic nerve-stimulated erection in dogs. American Journal of Phys 1993a; 264:H419-422.

Trigo-Rocha F, Hsu GL, Donatucci CF, Lue TF. The role of cyclic adenosine monophosphate, cyclic guanosine monophosphate, endothelium and nonadrenergic, noncholinergic neurotransmission in canine penile erection. Journal of Urology 1993b; 149:872-877.

Vaandrager AB, de Jonge HR. Signalling by cGMP-dependent protein kinases. Mol and Cell Biochemistry 1996; 157:23-30.

Yamakage M, Hirshman CA, Croxton TL. Sodium nitroprusside stimulates $Ca2^{+}$ activated K^{+} channels in porcrine tracheal smooth muscle cells. Amer. Journal of Phys. 1996; 270:338-345.

Yeh KH, Aoki H, Matsuzaka J, Ra S, Sato F, Fujioka T, Ohhori T, Kubo T, Yasuda N. Participation of vasoactive intestinal polypeptide as a humoral mediator in the erectile response of canine corpus cavernosum penis. Journal of Andrology 1994; 15:187-193.