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The Influence of Castration on Pharmacologically Induced Penile Erection in the Cat

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ABSTRACT: The purpose of this study was to investigate the *in vivo* effects of intracavernosal injections of adrenomedullin (ADM), calcitonin gene-related peptide (CGRP), nociceptin, vasoactive intestinal polypeptide (VIP), sodium nitroprusside (SNP), and prostaglandin E_1 (PGE_1) on penile erection in castrated and intact (control) anesthetized cats. Erectile responses to ADM, CGRP, nociceptin, VIP, SNP, and PGE_1 were compared with responses to a standard triple-drug combination (1.65 mg of papaverine, 25 μg of phentolamine, and 0.5 μg of PGE_1) in both castrated and control cats. In control animals, ADM, CGRP, nociceptin, VIP, SNP, and PGE_1 induced penile erections similar to those elicited by the triple-drug combination. However, in castrated animals, there was a significant decrease in erectile response; the response to intracavernosal injection of the standard triple-drug combination in castrated cats was 28% of that of the control group of animals. Serum testosterone levels demonstrated a significant ($P < 0.0001$) positive correlation ($r = 0.52$) with intracavernosal pressure in response to the standard combination. A marked reduction in serum testosterone levels was observed in castrated cats when measured by radioimmunoassay (0.34 ± 0.1 ng/dl in castrated cats, compared with 31.15 ± 6 ng/dl in control cats). These data suggest that the presence of testosterone is a necessary prerequisite to sustain a pharmacologically induced penile erection in the cat.

Key words: Testosterone, vasoactive agents, cAMP, cGMP.
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Normal sexual performance in males depends upon a complex interplay between psychological, neurological, vascular, and endocrine factors. Penile erection is a hemodynamic event involving increased arterial inflow and restricted venous outflow from the penis (Burnett, 1997). This process is generally accepted to be neuroregulated. Although the parasympathetic nervous system is involved in the erectile response, a nonadrenergic, noncholinergic (NANC) mechanism has been proposed to play a significant role (Anderson, 1993; Burnett, 1997). Diverse mediators (such as vasoactive intestinal polypeptide [VIP], calcitonin gene related peptide [CGRP], and adrenomedullin [ADM]) and other nonpeptide mediators (such as prostaglandin E_1 [PGE_1]) have been evaluated for their possible role as NANC neurotransmitters (Djamilian et al, 1993; Steif et al, 1993; Champion et al, 1997b). Nitric oxide (NO), a gaseous molecule, has been identified as both a central and peripheral mediator of penile erection in several species, including humans (Burnett et al, 1992; Ehmke et al, 1995; Lugg et al, 1995a). These mediators involve either cyclic guanosine monophosphate (cGMP) or cyclic adenosine monophosphate (cAMP) pathways to induce cavernosal smooth muscle relaxation, a prerequisite for the penile erection process (Ignarro et al, 1990; Trigo-Rocha et al, 1993; Lin et al, 1995; Miller et al, 1995).

The literature supports a significant role for androgens in normal male sexual function (Shabsigh, 1997). Androgen action in human males has conventionally been associated with libido; hence, the role of androgens in the regulation of the penile hemodynamic process is not well defined. Conversely, well-controlled laboratory investigations in animals, specifically in rat species, document a significant role for androgens in penile erectile mechanisms (Mills et al, 1992; Mills et al, 1994; Garban et al, 1995). The precise action of these steroids on penile smooth muscle function is a topic of current investigation.

Castrated rat models have been used by several investigators to examine the role of androgens in penile erection (Mills et al, 1994). Previous studies suggest that androgens may be important in the synthesis and action of neurotransmitters, specifically nitric oxide (Lugg et al, 1995b; Zvara et al, 1995). Androgen deprivation in the rat model has been shown to produce a decrease in the physiological neurogenic erectile response, most likely by affecting penile blood flow (Mills et al, 1994). Recent *in*

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vitro studies of isolated rabbit cavernosal muscle strips suggest that castration can affect the pharmacological smooth muscle response (Yildirim et al, 1997). However, the effect of castration on a pharmacologically induced erectile response has not yet been characterized in an *in vivo* model.

Therefore, this study, using the *in vivo* castrated feline model, was undertaken to gain insight into the role of androgens in cavernosal smooth muscle response to pharmacological stimulation. Vasoactive agents involving cGMP (sodium nitroprusside), cAMP (VIP and PGE_1), and other mechanisms of action (ADM, CGRP, and nociceptin) were administered intracavernosally to evaluate their interactions in comparison to a standard reference (a combination of papaverine, prostaglandin E_1, and phentolamine; Wang et al, 1994; Champion et al, 1997b). The plasma level of testosterone was correlated to the erectile response to the standard reference in order to delineate the role of testosterone on cavernosal smooth muscle relaxation.

Materials and Methods

Sixty normal adult male cats (3.2–5.9 kg) and 10 age-matched castrated adult male cats (3.2–6.0 kg) obtained from a USDA class B animal supplier were used in this study. The male cats that had undergone orchiectomy after puberty were supplied to our vivarial facilities by the animal supplier. The animals were sedated with ketamine hydrochloride (10–15 mg/kg intramuscularly) and anesthetized with pentobarbital sodium (30 mg/kg intravenously). Supplemental doses of sodium pentobarbital were administered as needed to maintain a uniform level of anesthesia. The animals were maintained at 37°C with a heating blanket. The trachea of each animal was cannulated, and the animals either spontaneously breathed room air or were ventilated with a Harvard model 607 respirator at a tidal volume of 40–60 ml at a rate of 15–22 breaths/minute. Catheters were inserted into the external jugular for intravenous administration of drugs and into the carotid artery for the measurement of systemic arterial (aortic) pressure. A vertical, circumcision-like incision was made to expose the two ventral corpora cavernosa and the dorsal corpus spongiosum. A 30-gauge needle was placed into the right corpus to permit administration of drugs into the penis. A 25-gauge needle was placed into the left corpus for the measurement of intracavernosal pressure. Systemic arterial and intracavernosal pressures were measured with Statham P23 transducers connected to a Grass model-7 polygraph, and mean pressures were obtained by electronic averaging. Techniques for the exposure of cavernosal tissue and recording of mean arterial and intracavernosal pressure were based on previously published articles (Wang et al, 1993; Champion et al, 1997a). Penile length was measured with a ruler. All procedures were approved by the Tulane University Animal Care and Use Committee.

Injections of agonists in both control and castrated animals were made when the cavernosal pressure was at a baseline value. The effect of a single injection of a randomized dose of an agonist on cavernosal pressure and penile length was measured until cavernosal pressure returned to the preinjection level. The next randomized injection was made at least 10–15 minutes after the previous intracavernosal response had returned to a stable baseline. Doses of agonists that produced modest erectile response in our previous studies were selected for use in this study. (Wang et al, 1993; Wang et al, 1994; Champion et al, 1997a; Champion et al, 1997b). An injection of 200 μl of saline vehicle had no significant sustained effect on intracavernosal pressure or penile length.

Blood samples were obtained through a catheter placed into the carotid artery. Aliquots of blood were placed into a 1.5-ml Eppendorf microcentrifuge tube and were spun down with an Eppendorf centrifuge to obtain blood serum. Serum testosterone levels were measured by Coat-A-Count radioimmunoassay (RIA) kit (Diagnostic Products, Los Angeles, California).

Calicitonin gene-related peptide, human ADM, VIP (Peptide Research Labs, Tulane University Medical School, New Orleans, Louisiana), nociceptin (orphanin FQ; Phoenix Pharmaceuticals, Mountain View, California), and sodium nitroprusside (SNP; Sigma Chemical Co., St. Louis, Missouri) were dissolved in 0.9% NaCl as separate solutions. All drug solutions were stored in a freezer in amber bottles; working solutions were prepared on a frequent basis and kept on crushed ice. The agonists were administered intracavernosally in small volumes (200 μl) in both control and castrated animals. The standard triple-drug combination comprised PGE_1 (0.5 μg; Upjohn Pharmaceuticals, Kalamazoo, Michigan), papaverine (1.65 mg), and phentolamine (25 μg; Sigma) was prepared and injected intracavernosally at the end of each experiment to serve as a control comparison for all erectile agents studied as previously described (Hellstrom et al, 1992).

The data were expressed as means ± standard error of the mean (SEM) and were analyzed by Student's *t*-test for single-group comparison and by one-way analysis of variance (ANOVA) with Tukey's test for multiple-group comparisons. A P value of less than 0.05 was established as the criterion for statistical significance.

Results

Comparison of Responses in Control and Castrated Cats

The effects of intracavernosal injections of ADM (1 nmol/ml), CGRP (0.3 nmol/ml), nociceptin (10 nmol/ml), VIP (0.3 nmol/ml), SNP (10 μg/ml), PGE_1 (0.3 μg/ml), and a triple-drug combination (1.65 mg papaverine, 25 μg phentolamine, and 0.5 μg PGE_1) on intracavernosal pressure and penile length in control and castrated cats are illustrated in Figures 1 and 2. In the control animals, ADM, CGRP, nociceptin, VIP, SNP, and PGE_1 induced penile erections that were comparable to the erectile response elicited by the standard triple-drug combination (Fig. 1). However, in the castrated animals, a significant decrease was observed in the erectile response to intracavernosal injections of these agents when compared to

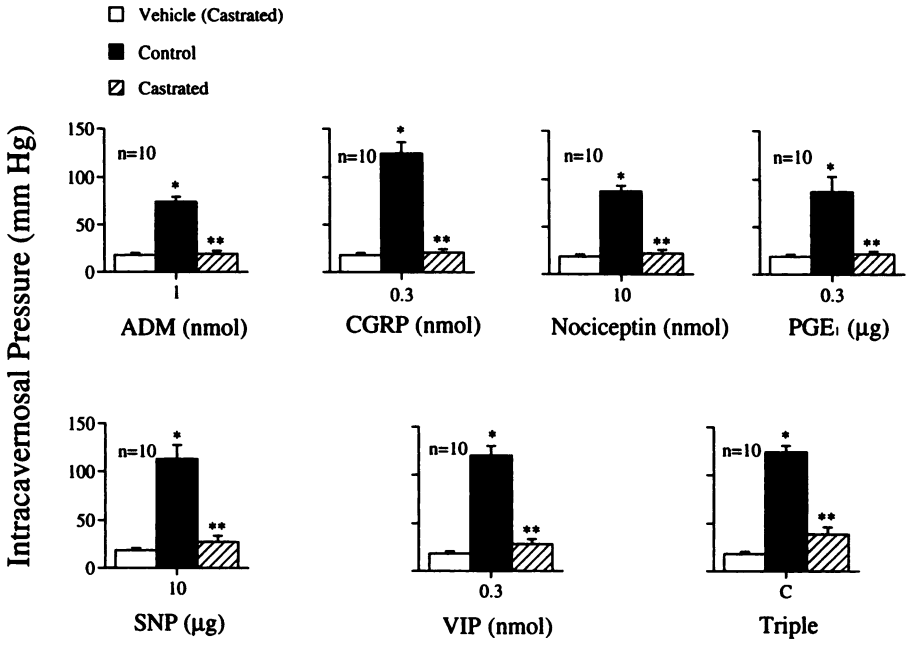

FIG. 1. Bar graphs showing cavernosal pressure in control and castrated cats in response to intracavernosal injections of ADM, CGRP, nociceptin, VIP, SNP, PGE_1, and triple-drug combination. *Triple* denotes control triple-drug combination (papaverine, phentolamine, and PGE_1) administered at the end of the experiment; *n* indicates the number of animals. One asterisk indicates that the response is significantly different from the baseline; two asterisks indicate that the response is significantly different from the response elicited by the agents in control cats. * $P < 0.05$.

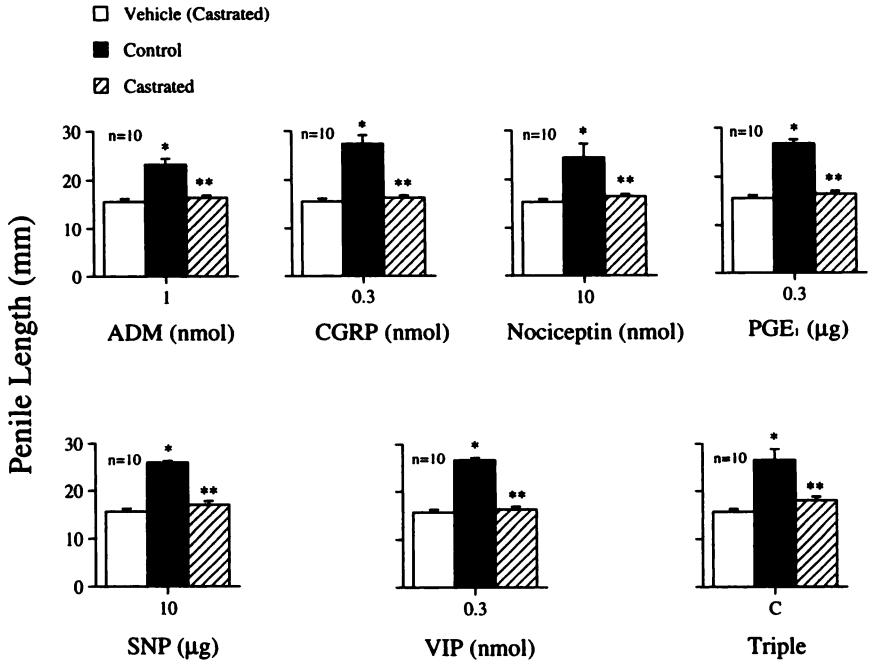

FIG. 2. Bar graphs showing penile length in control and castrated cats in response to intracavernosal injections of ADM, CGRP, nociceptin, VIP, SNP, PGE_1, and triple-drug combination. *Triple* denotes control triple-drug combination (papaverine, phentolamine, and PGE_1) administered at the end of the experiment; *n* indicates the number of animals. One asterisk indicates that the response is significantly different from the baseline; two asterisks indicate that the response is significantly different from the response elicited by the agents in control cats. * $P < 0.05$.

Table 1. *Decrease in mean systemic arterial pressure (mm Hg) in response to intracavernosal injection in control and castrated cats*

Drug administered	Control (mm Hg)	Castrated (mm Hg)
ADM (1 nmol) (n = 10)	−10.0 ± 4.3	−6.7 ± 0.8
CGRP (0.3 nmol) (n = 10)	−29.8 ± 8.2	−20.0 ± 10.2
Nociceptin (10 nmol) (n = 10)	−17.3 ± 4.5	−14.3 ± 2.5
PGE_1 (0.3 μg) (n = 10)	−5.9 ± 2.1	−5.5 ± 2.6
SNP (10 μg)* (n = 10)	−16.4 ± 1.4	−15.0 ± 2.7
VIP (0.3 nmol) (n = 10)	−10.0 ± 1.5	−8.3 ± 3.3
Triple-drug combination† (n = 10)	−35.8 ± 4.2	−37.5 ± 5.3

Values are mean ± SEM; n indicates the number of animals.
* Nitric oxide donor.
† Other combination of 1.65 mg papaverine, 25 μg phentolamine, and 0.5 μg prostaglandin E_1.

Table 2. *Duration of erectile response to intracavernosal injection in control and castrated cats*

Drug administered	Control (minutes)	Castrated (minutes)
ADM (1 nmol) (n = 10)	15.6 ± 4.1	0.0 ± 0.0
CGRP (0.3 nmol) (n = 10)	13.5 ± 3.4	0.0 ± 0.0
Nociceptin (10 nmol) (n = 10)	24.5 ± 5.4	0.4 ± 0.3
PGE_1 (0.3 μg) (n = 10)	14.0 ± 5.3	0.0 ± 0.0
SNP (10 μg)* (n = 10)	9.3 ± 2.9	2.5 ± 1.1
VIP (0.3 nmol) (n = 10)	6.0 ± 1.0	1.1 ± 0.5
Triple-drug combination† (n = 10)	43.7 ± 8.2	9.3 ± 3.5

Values are mean ± SEM; n indicates the number of animals.
* Nitric oxide donor.
† Other combination of 1.65 mg papaverine, 25 μg phentolamine, and 0.5 μg prostaglandin E_1.

the control cats (Fig. 1). The baseline cavernosal pressure in the castrated animals (18.2 ± 2.4 mm Hg) was significantly decreased when compared to that of the controls (27.1 ± 1 mm Hg). A slight increase in cavernosal pressure was observed after intracavernosal injections of ADM (19.5 ± 3.3 mm Hg), CGRP (21.2 ± 3.7 mm Hg), nociceptin (22 ± 3.7 mm Hg), PGE_1 (21.2 ± 3 mm Hg), SNP (27.5 ± 6.3 mm Hg), and VIP (28.1 ± 5.5 mm Hg). The triple-drug combination increased cavernosal pressure to 40 ± 7.3 mm Hg from a baseline of 18.2 ± 2.4 mm Hg in castrated animals. The order of potency of these agents in castrated cats was as follows: (1) triple-drug combination > (2) VIP > (3) SNP > (4) nociceptin > (5) PGE_1 > (6) CGRP > (7) ADM. There was no significant increase in penile length in castrated cats after any intracavernosal injection of these agents, as compared to the significant increase of penile length observed in the control group of cats (Fig. 2).

Effects on Systemic Arterial Pressure

Intracavernosal injections of ADM (1 nmol/ml), CGRP (0.3 nmol/ml), nociceptin (10 nmol/ml), VIP (0.3 nmol/ml), SNP (10 μg/ml), PGE_1 (0.3 μg/ml), and the triple-drug combination (1.65 mg papaverine, 25 μg phentolamine, and 0.5 μg PGE_1) produced a significant decrease in systemic arterial pressure in both control and castrated animals (these data are compared in Table 1). Basal systemic arterial pressure was similar in both groups of animals. Decreases in systemic arterial blood pressure in response to these vasoactive agents were similar in both control and castrated cats (Table 1). The decrease in systemic arterial pressure induced by all agents abated, and pressure returned to a baseline value over a 5- to 30-minute period, depending on the efficacy of the intracavernosal agent administered (data not shown). The decrease in systemic arterial pressure in response to ADM (1 nmol/ml), CGRP (0.3 nmol/ml), and PGE_1 (0.3 μg/ml) was smaller in magnitude when compared to other vasoactive agents, such as VIP (0.3 nmol/ml), SNP (10 μg/ml), and nociceptin (10 nmol/ml; Table 1). In both groups of animals, the triple-drug combination induced the largest decrease in systemic arterial pressure when compared to all the other individual agonists (Table 1).

Duration of Erectile Response

The duration of erectile response to various vasoactive agents is summarized in Table 2. In control animals, the duration of erectile response to erectogenic agents ranged from 6.0 ± 1.0 minutes to 43.7 ± 8.2 minutes, in comparison to 0 minutes to 9.3 ± 3.5 minutes in castrated cats (Table 2). Castrated cats exhibited a 70%–90% decrease in duration of response to nociceptin, SNP, VIP, and the triple-drug combination when compared to the control group of cats. The order of potency in terms of erectile response in castrated cats was as follows: (1) triple-drug combination > (2) SNP > (3) VIP > (4) nociceptin > (5) ADM, CGRP, and PGE_1. The duration of erectile response to the triple-drug combination in castrated cats was longer when compared to individual agents, such as ADM, CGRP, nociceptin, VIP, SNP, and PGE_1 (Table 2).

Testosterone RIA

Serum plasma testosterone levels were measured using RIA. Mean plasma levels of testosterone in control and castrated animals were 31.15 ± 6 ng/dl and 0.34 ± 0.1

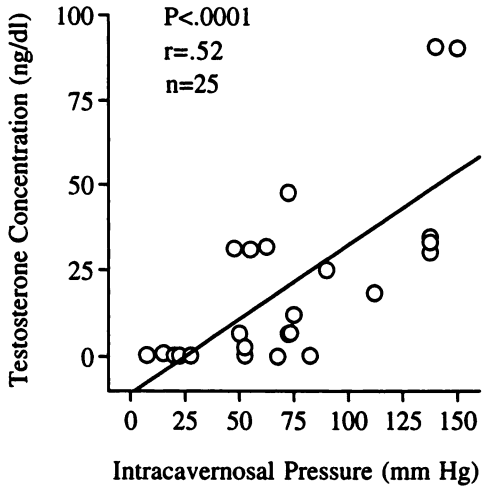

FIG. 3. Scattergram graph showing the correlation of serum testosterone levels (ng/dl) with the increase in intracavernosal pressure elicited by the standard triple-drug combination (mm Hg) in control and castrated cats; *n* indicates the number of animals.

ng/dl, respectively. A significant ($P < 0.001$) positive correlation ($r = 0.52$) was observed between the levels of plasma testosterone and the erectile response to the triple-drug combination in castrated and control cats as measured by intracavernosal pressure (Fig. 3).

Discussion

In this study, we compared the *in vivo* pharmacological erectile response to intracavernosal administration of erectogenic agents in castrated and intact cats. The feline model used in our laboratory is a well-accepted animal model for investigation of the physiology and pharmacology of penile erectile function (Hellstrom et al, 1992; Wang et al, 1993; Champion et al, 1997a; Champion et al, 1997b). Available evidence suggests that the hemodynamic events preceding the erectile response in the anesthetized cat are similar to those in humans, due to the afferent anatomical and physiological similarities between cats and men (Root and Bard, 1947; Domer et al, 1978). As in humans, the two corpora cavernosa in the cat communicate; therefore, a vasoactive agent can be injected into one corpora cavernosa, and penile pressure can accurately be measured in the other. Previous studies have shown that the male cat responds to vasoactive agents, which induce an erection through either stimulating soluble guanylate cyclase or through increasing cAMP levels (Wang et al, 1993, 1994). These observations suggest the involvement of both cGMP- and cAMP-dependent mechanisms in the feline erectile response. In the present study, castrated cats demonstrated a functional reduction in response to all the intracavernosal pharmacological and vasoactive agents as compared to normal intact cats. There was a positive correlation between the levels of testosterone in plasma and the intracavernosal pressure response to the standard triple-drug combination.

The mechanisms underlying the relaxation of cavernosal smooth muscle are not fully understood. However, the release of NANC agents seems to play a significant role in this process. The vasoactive agents employed in this study have previously been shown to cause erections in cats. PGE_1 and VIP act via an increase in cAMP to induce vasorelaxation of cavernosal smooth muscle, leading to an erection (Andersson et al, 1987; Wang et al, 1993). Nitric oxide donors involve cGMP-dependent mechanisms to induce erectile response in the cat (Wang et al, 1994). Neuropeptides, like adrenomedullin and calcitonin gene-related peptide, have been shown to cause penile erection in the cat by mechanisms other than NO or the opening of K^+_{ATP} channels (Champion et al, 1997b). Recently, another neuropeptide, nociceptin, has also been reported to cause erectile response in the cat through a naloxone-insensitive mechanism (Champion et al, 1997a).

In intact cats, all agents studied induced an increase in intracavernosal pressure and penile length when injected directly into the corpora cavernosa. This erectile response to vasoactive agents was reproducible, suggesting that it was mediated by a local effect at the level of the corpora cavernosa. However, in castrated cats, when these same erectogenic agents were directly injected into the corpora cavernosa, the response was attenuated significantly. Previous studies in rats have demonstrated some residual erectile response after castration. However, some of the agonists used in this study did not elicit such a phenomenon. The lack of such an effect in cats to drugs in this study may be attributed to postcastration duration or to the altered sensitivity of cavernosal smooth muscle to androgen depletion. These findings suggest that castration significantly impairs the erectile response to all erectogenic agents possibly by affecting the hemodynamic mechanism. Conversely, Lin et al (1990) found in the castrated dog that castration does not affect the erectile mechanism and that the reduction in cavernosal pressure is attributable to decreased systemic arterial pressure. The attenuation of erectile response in castrated cats was not due to decreased systemic arterial pressure, since this specific event was not observed. The significant decrement in the erectile response to these vasoactive agents in the castrated cats suggests that one or more androgen-mediated events were compromised.

Androgen deprivation has been demonstrated to produce a multitude of effects in the penis (Shabsigh, 1997). In rats, castration compromised the veno-occlusive mechanism and attenuated the erectile response (Mills et al, 1994). In both rats and cats, the striated muscles, such as the bulbocavernosus and ischiocavernous muscles, were

reduced in size after androgen depletion (Leipheimer and Sachs, 1993; Hughes et al, 1983). In a detailed analysis of the effect of castration on neural afferent responses in the domestic cat, Cooper and Aronson (1974) demonstrated that decreases in the androgen level affected the physical properties of the penile integument, at least in part by a decrease in peripheral receptors. Serum testosterone levels have been measured in control and castrated cats to ascertain the actual decrease in testosterone levels. The results demonstrate that testosterone levels were significantly diminished in castrated cats compared to intact cats, which suggests that testosterone has a direct effect on cavernosal smooth muscle and supports the precept that testosterone maintains the responsiveness of cavernosal smooth muscle for the purpose of penile erection.

The role of androgens in human male sexual function is indirectly derived from clinical experience with surgically castrated sex offenders and men suffering from endocrine disorders. Studies show that castration may cause a decrease in libido and diminished erectile ability but that some castrated men are still able to engage in sexual intercourse (Spark et al, 1980; Heim, 1981; Baskin, 1983). The implications from these studies are difficult to ascertain, due to the lack of objective assessment of erectile dysfunction. In clinical studies, it is difficult to separate libido from erectile function at the level of the penile erectile tissue; therefore, it is difficult to find a cause–effect relationship between androgen deprivation and erectile dysfunction.

In summary, results from this study provide additional evidence in support of the hypothesis that corporeal responsiveness to pharmacological stimulation may be an androgen-dependent mechanism in some species. Overall, these studies suggest that testosterone maintains the responsiveness of cavernosal smooth muscle of the penis to pharmacological stimulation. We have employed various vasoactive agents in an established animal model to identify changes in specific physiological pathways. Intracavernosal injections of ADM, CGRP, nociceptin, VIP, SNP, and PGE_1 produced increases in cavernosal pressure in control cats, while intracavernosal injections of these agents in castrated cats caused little, if any, erectile response. The role of testosterone in the maintenance of nerve-stimulated erectile response has been explored in rat models; however, this is the first study to show the correlation of testosterone to penile erection when induced by pharmacological stimulation (Mills et al, 1994). Additional studies are necessary to further address the mechanism of androgen regulation in penile erection.

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