Effects of Castration and Androgen Replacement on the Hemodynamics of Penile Erection in the Rat¹

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ABSTRACT

Previous studies from this laboratory have demonstrated that penile erection in the rat is androgen dependent: 1 wk after castration, there was a significant decline in the magnitude of the intracavernosal pressure (CCP) response during erection induced by stimulation of the autonomic ganglion controlling penile blood flow. The response was altered by vasoactive drugs and appeared to involve nitric oxide synthesis. These earlier studies, however, did not identify the site of androgenic action or the mechanism by which the andro gens act. The findings reported here show that even in long-term-castrated animals (up to 7 wk), there remains a rise in CCP in response to ganglionic stimulation, demonstrating that there is an androgen-independent as well as an androgen-dependent portion of the erectile response. Other results show a linear relationship between systemic blood pressure and CCP during erection, although in castrated animals without androgen replacement, the CCP responds less to changes in the systemic pressure than in intact or testosterone-treated animals. This finding could signify a reduced blood inflow and/or an increased blood outflow during erection in the castrated rats. Further studies partially explained the lower erectile pressure by demonstrating that the rate of outflow from the cavernosal spaces was greater in castrated rats than in animals with normal androgen levels. Taken together, these findings show that androgens act to maintain both the inflow and the outflow of blood from the cavernous spaces during erection.

INTRODUCTION

Earlier studies from this and other laboratories have shown that the rise in intracavernosal pressure (CCP) during penile erection follows increased inflow and accumulation of blood in the cavernous spaces of the penis [1–3]. In many species, the pressure rise in the erectile tissue is diminished by treatment with an inhibitor of the enzyme nitric oxide synthase (NOS), supporting the conclusion that nitric oxide is a major neurotransmitter of erection in men [4–6], rabbits [7–9], dogs [10], and rats [11–13]. Other findings reveal that the increase in CCP during erection is markedly higher in castrated animals treated with testosterone than in untreated castrated animals, demonstrating that penile erection in the rat is an androgen-dependent process [12]. In other species, however, the role of androgens is less clear [14–17]. In ongoing experiments in this laboratory, the basic mechanisms of penile erection in the rat and the androgen maintenance of the process are being investigated. The present studies were designed to 1) determine whether changes in the mean arterial pressure influence CCP during erection and whether androgens are involved, and 2) determine whether the rate of blood flow out from the cavernous spaces is elevated in castrated animals and whether higher outflow is responsible for the lowered pressure during erection in these animals.

MATERIALS AND METHODS

Animals

Male rats (of the Harlan-Holtzman strain), ranging in age from 80 to 240 days, were castrated under ether anesthesia by standard methods. At the time of the castration, 5-mg pellets of testosterone (group designation: TESTO) or pellets of cholesterol (control group designation: CASTRATE) were implanted under the skin of the dorsal neck region. In some experiments, noncastrated (group designation: INTACT) rats were also used. In some rats, CCP measurements were made 1 wk after castration, while other rats were left untreated for up to 7 wk after castration before measurement of cavernosal pressure.

Measurement of the CCP

The procedure used to measure CCP has been reported previously [12]. In the present study, which employed a modification of the method of Quinlan et al. [18], rats were anesthetized with intramuscular ketamine (87 mg/kg) plus xylazine (13 mg/kg) and maintained on supplemental ketamine as needed. The anesthetized rat was placed on a heating pad, and the left carotid artery was cannulated with a blunt 18- or 21-gauge needle attached to saline-filled PE 200 tubing that was connected to a pressure transducer to continuously monitor mean arterial blood pressure (MAP). In some studies, an additional cannula was inserted into the right jugular vein for the systemic infusion of vasoactive drugs. The abdominal cavity was then opened, and the viscera were retracted and wrapped in warmed saline-soaked sponges and cellophane wrap to reduce evaporation and help maintain body temperature. The right major pelvic ganglion was exposed by clearing the overlying fascia but
was not decentralized by cutting the afferent innervation. Once the cannulae were in place and the ganglion was exposed, the shaft of the penis was dissected free of skin and fascia, exposing the paired corpora cavernosa distal to the crura. The right corpus cavernosum was cannulated by insertion of a 30-gauge needle attached to PE 200 tubing drawn to a fine tip. This cannula was also saline filled and attached to a pressure transducer. Stainless steel bipolar electrodes were positioned on the major pelvic ganglion via a micro-manipulator and connected to a stimulator for delivery of bipolar electrical stimulations. During the experiments, MAP, CCP, time, frequency, and amplitude of stimulation were continuously recorded. Stimulatory voltage was varied from 1 to 6 V (duration, 5 msec; frequency, 12 Hz), and the cavernosal pressure response at each voltage was compared to determine the threshold voltage and the voltage required for maximal response. The duration of each stimulation was 1 min, and there was a 2-min rest period between subsequent stimulations. At the end of the experiment, blood was collected for the measurement of circulating levels of testosterone by RIA as routinely performed in this laboratory [12]. In each experiment, the pressure transducers were calibrated with a mercury manometer prior to use.

**Determination of the Effects of Androgens on the Relationship between the Systemic Blood Pressure and the Pressure in the Cavernous Spaces**

While the CCP was continuously monitored during induced erection in INTACT, TESTO, and CASTRATE animals, MAP was varied in either of two ways: by infusing 10 µg nitroglycerin/kg/min into the left jugular vein or by passing a ligature under the right carotid and elevating it to halt blood flow to the carotid sinus baroreceptors, leading to increased systemic blood pressure (note: the left carotid was occluded by the cannula). Changes in the response of the cavernosal vasculature were demonstrated by plotting a wide range of MAP values against the CCP measured at the same time point.

**Determination of the Effects of Androgens on the Cavernosal Outflow Rate from the Cavernous Spaces**

Rats in the CASTRATE and TESTO groups received two cannulae: one inserted into the right and the other into the left cavernous space. One cannula was connected to a pressure transducer for continuous measurement of CCP while the second, consisting of PE 50 tubing attached to a 29-gauge needle, was connected to a variable-speed syringe pump for infusion of saline into the cavernous spaces (infusion rates ranged from 0.05 ml/min to 1.0 ml/min). During the experiment, the infusion rate was increased step-wise for periods of 1–3 min until the pattern of CCP rise indicated that the infusion rate had exceeded the outflow rate and CCP rose sharply.

**Statistical Analysis [19]**

Data in the present studies were analyzed by ANOVA or Student's t-test when only two means were compared. Post hoc comparisons between means were made by Newman-Keuls test. Linear regression analysis was used to compute best fit lines.

**RESULTS**

Seven to nine days after castration, blood levels of testosterone had declined from an average of 1960 ± 280 pg testosterone/ml serum in INTACT rats to 20 ± 2 pg/ml in CASTRATE animals. Seven days after rats were implanted with a single pellet of testosterone at the time of castration (TESTO), the mean blood level of the androgen was 1070 ± 140 pg/ml.

Figure 1 shows the erectile response in INTACT, CASTRATE, and TESTO animals expressed as the ratio of CCP to MAP. This method of expressing the results is used since electrical stimulation of the major pelvic ganglion leads to a small, transitory decline in MAP in some animals. This figure shows the marked difference in magnitude between the response in CASTRATE animals and that in TESTO and INTACT animals.

Previous studies from this laboratory have demonstrated that the magnitude of the erectile response declines measurably by 24 h postcastration and that the decline continues through 1 wk after removal of the testes. The results in Figure 2 show that there is a slight additional decrease in the response up to 7 wk after castration but that even in these long-term-castrated animals, a portion of the erectile response remains.

In studies designed to measure the effect of changes in systemic pressure on CCP, MAP was varied by infusing a
vasodilator (nitroglycerin) or by temporarily interrupting blood flow to the baroreceptors in the carotid sinus by occluding the carotid artery. The effects of these manipulations on MAP were variable, but overall, nitroglycerin caused a decrease of 6–48 mm Hg in MAP while carotid occlusion caused an increase of 6–50 mm Hg in MAP. The effect of MAP on CCP (Fig. 3) demonstrates that during erection, intracavernosal and peripheral pressures are related in a linear fashion. In the INTACT, TESTO, and CASTRATE animals, there is a significant correlation between MAP and CCP over a wide range of MAP values ($r = 0.94$, $0.95$, and $0.63$, respectively, $p < 0.05$). When the slopes of the regression lines are compared, it is clear that the responses in the INTACT and TESTO groups are similar to one another but different from the response observed in the CASTRATE animals. In castrated rats, the rise in CCP was significantly less (relative to MAP) than the CCP rise in the TESTO and INTACT animals.

A second set of studies was done to determine whether the observed androgen-dependent change in the erectile response was due to differences in the rate at which blood flowed out of the cavernous spaces. We reasoned that a higher rate of blood flow out of the cavernous spaces in the CASTRATE rats than in the TESTO animals could account for the lower CCP during erection in the CASTRATE. In each of the animals, the rate of saline infusion into the cavernous spaces was increased step-wise until the rate of rise in CCP indicated that the outflow capacity of the vessels had been exceeded and the pressure rose rapidly (Fig. 4). The results of these studies, presented in Figure 5, demonstrate that the rate of outflow in CASTRATE rats was significantly greater than in the TESTO animals ($p < 0.05$).

**DISCUSSION**

The results presented in Figure 2 demonstrate that even after a 7-wk absence of androgen, the CCP still increases in response to stimulation of the major pelvic ganglion. The finding that there is not a complete loss of erection in chronically castrated animals may mean that there is an androgen-dependent portion, accounting for 60–70% of the response, and an androgen-independent response accounting for the remaining 30–40%. The significance of this finding may relate to the fact that penile erection in the rat is dependent on the synthesis and release of nitric oxide [11–13], the neurotransmitter synthesized by the enzyme NOS.
Several types of NOS, including constitutive forms and inducible forms, have been reported [6, 20–23]. A variety of agents have been found to induce the formation of this enzyme, including endotoxins, interleukins, and L-arginine [21]. Thus, we propose that in the cavernosal system of the rat, testosterone is the agent that induces and maintains NOS activity, accounting for the 60–70% of the erectile response under androgenic control.

The importance of the contribution of MAP to the magnitude of the erectile response was investigated in the studies presented in Figure 3. In this experiment, MAP was varied over a wide range and the corresponding changes in CCP during erection were measured. While there was no difference in the effect of carotid occlusion and vasodilator on MAP in INTACT, CAstrate, and TESTO animals, the increase in CCP in the CAstrate rats was significantly less than in the INTACT and TESTO animals. This finding presents three possibilities. First, the CAstrate group may have a diminished response to stimulation in the cavernosal arteries such that less blood flows into the cavernous spaces and the CCP rise is reduced. Secondly, in the CAstrate group the outflow may be greater so that less blood accumulates in the spaces and the CCP is lower. The third possibility is that the cavernosal vasculature is more compliant in the castrated animals, possibly because of the loss of collagen [24].

To determine whether androgens are involved in regulating the outflow of blood from the cavernous spaces, saline was infused into the cavernous spaces at increasing rates until the inflow rate exceeded the outflow rate and CCP rose sharply (Fig. 4). As shown in Figure 5, the inflow rate required in the CAstrate group was nearly twice that required in the TESTO animals. This finding shows that androgens regulate the magnitude of the erectile response, in part, by regulating the venous outflow from the cavernous spaces. The route of venous outflow from the cavernous spaces has recently been described by Fernandez and coworkers [25] on the basis of scanning electron microscopic examination of corrosion casts of the vasculature of the rat penis. The study revealed that blood drains from the cavernous spaces into the cavernous vein, which drains blood proximally along the dorsal side of each cavernosum. In the crus region, the cavernous vein divides into multiple parallel channels that rejoin to form the two crural veins and pass through the tunica albuginea and out of the penis. On the basis of this arrangement and the absence of circular smooth muscle in the walls of these veins, the authors conclude that active smooth muscle contraction apparently does not play a regulatory role in the flow of blood out of erectile tissue. Rather, the angioarchitecture of the system suggests the involvement of a mechanical venous occlusion mechanism in which the expansion of blood in the cavernous spaces during erection partially occludes cavernous channels by compressing them against the tunica albuginea. On the basis of the results of the present study, at least a portion of this occlusive mechanism can be said to be androgen dependent.

The increased outflow of blood from the cavernous spaces in the penis of the castrated rat resembles the clinical syndrome of venous impotence in many respects [26] and has been used as an animal model for venous impotence [27]. In both situations, a failure to trap an adequate volume of blood in the cavernous spaces results in reduced CCP and lack of rigidity in the penis. In men, the problem may result from fistulae or from Peyronie's disease, although in many men with venous impotence, there is no apparent pathol-
ology in the venous system. Wespes and Schulman [26] theorize that dysfunction of the corpora smooth muscle or deterioration of the fibroelastic elements of the cavernosal trabeculae may be the underlying cause of this form of impotence. Whatever the cause in men, the diagnosis of venous impotence is confirmed by intracavernosal saline infusion accompanied by continuous monitoring of CCP. If an infusion rate greater than 15 ml/min is required to maintain the penis in an erect state, the patient is diagnosed as having impotence due to caverno-venous leakage. In the present rat study, the rate of saline infusion into the cavernous spaces had to be twice as great in CASTRATE rats as in TESTO rats to achieve a CCP of 60 mm Hg. The underlying basis for the increased rate of outflow of blood from the cavernous spaces in castrated animals is unknown. Our earlier finding that the magnitude of the response is significantly improved in castrated rats within 24 h of androgen treatment [12] argues against the high flow rate being based on a structural change in the penile angioarchitecture. It is unlikely that significant remodeling of the cavernosal structure could occur in such a short period of time. Furthermore, as cited above, Fernandez and coworkers [25] reported that the presence of little vascular smooth muscle in the cavernous veins or associated vessels could rule out decreased vascular tone as the reason for the reduced CCP during erection in the castrated animals.

REFERENCES