Effects of castration on adrenergic, cholinergic and nonadrenergic, noncholinergic responses of isolated corpus cavernosum from rabbit

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Objective To investigate the effects of castration and testosterone on the constricting effect of phenylephrine and endothelium-dependent and -independent relaxing effects of different agonists in the corpus cavernosum of male rabbits.

Materials and methods Twenty rabbits were castrated and 10 received testosterone replacement for 1 month after castration; 10 further rabbits underwent a sham operation and acted as controls. One month after operation the rabbits were killed and their penises excised. Strips of corpus cavernosum were used for isometric tension measurements in organ chambers; concentration-response relationships for phenylephrine, carbachol, adenosine and sodium nitroprusside were obtained by adding the reagent cumulatively to the bath.

Results The phenylephrine-induced contractions were markedly lower, with no change in the pD₂ values (i.e. the negative logarithm of the concentration for half-maximal response), in cavernosal strips obtained from castrated rabbits than in those from controls.

Conclusion The lack of testosterone has an effect on the reactivity of the corpus cavernosum, indicating that testosterone has an important role in erectile function and that its loss impairs the ability of the corpus cavernosum to relax in response to certain stimuli.

Keywords Corpus cavernosum, castration, testosterone, contraction, relaxation, rabbit penis

Introduction Androgens are essential for male sexual maturity [1] and castration produces loss of both libido and potency in man and animals [2,3]. Although impotence is a major clinical problem in men, studies have shown that erection in response to visual sexual stimulation is not affected by androgen withdrawal in hypogonadal men, suggesting that androgen enhances but is not essential for erection [4,5]. Androgens are known to act on the hypothalamus, an important site for the modulation of erectile function. However, the local effects of castration and the resulting low level of testosterone are still unclear and controversial [6–8].

Penile erection probably depends on parasympathetic neurally mediated, smooth-muscle relaxation in the cavernosal arterial venous bed and trabecular network [9]. During erection, relaxation of trabecular smooth muscle allows the expansion of the lacunar spaces and compression of subtunical veins, with entrapment of blood in the corpora. This process is controlled by autonomic dilator nerves and the corporeal endothelium [10]. Recent studies suggest that nitric oxide (NO) mediates both neurogenic and endothelium-dependent relaxation of the trabecular smooth muscle [11–13]. The role of NO in erection and erectile dysfunction is probably multifactorial and complex. For detumescence, the smooth muscle of penile arteries and erectile tissue must contract and this contraction is probably mediated mainly by the release of norepinephrine acting on post-junctional alpha adrenoceptors [14–16]. Recent in vitro studies have indicated that the cause of erectile dysfunction in many patients may be related to increased contractility or impaired relaxation of corporal smooth muscle [17–19].

Thus, we examined whether castration and testosterone-replacement therapy affect the neurogenic,
endothelium-dependent and -independent relaxation and phenylephrine-induced contractile responses of corpus cavernosal smooth muscle in the rabbit penis.

Materials and methods

Mature male albino rabbits (body weight 2.5–3 kg) were chosen as the animal model because close similarities have been reported in the reactivity of human and rabbit corpus cavernosum in vitro [20]. The animals were divided into three groups; group 1 served as controls and underwent a sham-operation, groups 2 and 3 were castrated 1 month before death and group 3 were treated with intramuscular testosterone propionate (10 mg/day) during the month after castration. Orchidectomy was performed in aseptic conditions under anaesthesia induced by subcutaneous ketamine (50 mg/kg) and xylazine (5 mg/kg). In groups 2 and 3, an incision was made along the midline of the scrotum, both vasa and spermatic cords were ligated, the testes were removed and the wound closed. In the control group, the testes were mobilized but left intact.

One month later the rabbits were killed with a subcutaneous injection of ketamine and xylazine, exsanguinated and the penis removed. Immediately after removal, the specimens were placed in Krebs-bicarbonate solution (in mmol/L: NaCl 118, KCl 4.7, CaCl\(_2\) 2.5, NaHCO\(_3\) 1.25, MgSO\(_4\) 1.2, KHPO\(_4\) 1.2, glucose 11) and all surrounding tissue was removed by sharp dissection.

Organ-chamber measurements

Strips of cavernosal tissue (2 × 2 × 15 mm) were used to obtain isometric tension measurements in 20 mL organ chambers. The strips were tied with silk to a force transducer (Grass PT 03, Quincy, MA, USA) on one end and fixed with silk ties to a glass support on the other. The organ chambers contained Krebs-bicarbonate solution, gassed with 95% O\(_2\) and 5% CO\(_2\) during the measurements, and maintained at 37°C. After mounting, the preparations were allowed to equilibrate for 1 h during which the tension was adjusted to 2 g. At the end of the equilibration, strips were depolarized with 124 mmol/L KCl in Krebs-bicarbonate solution and allowed to equilibrate for 30 min. This procedure increases and stabilizes subsequent contractile responses to phenylephrine and decreases spontaneous contractions. After equilibration, the contractile responses to phenylephrine (10\(^{-5}\)–10\(^{-4}\) mol/L) were obtained cumulatively; after adding each dose, a plateau response was obtained before adding the next. On completing the phenylephrine concentration-response curve, tissues were washed for a further 30 min and pre-contracted with a submaximal concentration of phenylephrine (10\(^{-5}\) mol/L). After this contraction stabilized, concentration-response relationships were obtained for carbachol (10\(^{-9}\)–10\(^{-4}\) mol/L), adenosine (10\(^{-6}\)–10\(^{-4}\) mol/L) and sodium nitroprusside (10\(^{-8}\)–10\(^{-5}\) mol/L) by adding one of the agents cumulatively to the bath. Isometric tension was recorded on a Grass model 79E polygraph.

Electrical-field stimulation (EFS)

The strips were stimulated for 10 s with two parallel platinum electrodes at sequential frequencies of 2, 4, 8, 16 and 32 Hz as square-wave pulses of 50 V (0.8 ms) delivered by a current amplifier and a stimulator (S 88, Grass). The strips were allowed to return to the baseline pre-contraction tension between the tests at each frequency. Subsequently, without the strips being washed, N-nitro l-arginine methyl ester (L-NAME; 3 × 10\(^{-5}\) mol/L) was added to the chamber and the stimulation was repeated 15 min later. Before EFS, the tissue was treated with an adrenergic nerve blocker, guanethidine (5 μmol/L) and a muscarinic receptor blocker, atropine (1 μmol/L) for 30 min to eliminate the adrenergic and cholinergic responses and to study relaxation responses induced by stimulation of nonadrenergic, noncholinergic nerves.

Analysis

Contractile responses to phenylephrine were calculated as a percentage of the maximal contraction caused by potassium (124 mmol/L). The relaxant effects of agonists were expressed as a percentage of the pre-contraction to phenylephrine. To evaluate the effects of agonists the maximum response (Em) and pD2 values (i.e. the negative logarithm of the concentration for the half-maximal response, ED\(_{50}\)) were calculated. The concentration-response data obtained in each experiment were plotted as the response/concentration against the response, producing a linear relationship in each experiment, as predicted from the Scatchard equation for drug-receptor interaction. Agonist pD2 values (apparent agonist affinity constants) were calculated from each agonist concentration-response curve by linear regression of the linear part of the curve and taken as a measure of the sensitivity of the tissues to each agonist. Groups were compared statistically using general linear models of ANOVA followed by Scheffe’s F-test. Values of P < 0.05 were considered to indicate statistical significance.

Drugs

The following drugs were used: adenosine, acetycholine chloride, papaverine hydrochloride, phenylephrine hydrochloride, L-NAME, sodium nitroprusside, atropine

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sulphate, guanethidine sulphate, carbachol chloride and testosterone propionate (Sustanon, Organon, USA). L-NAME was initially dissolved in distilled water and stored frozen; on the day of use it was thawed and diluted in distilled water. All other drugs were prepared daily.

Results

The cumulative addition of phenylephrine produced concentration-dependent contractions of the cavernosal strips and the contractility was significantly lower in the castrated rabbits \((P < 0.05)\). The concentration-response curve for phenylephrine was shifted to the right, with significantly lower \(Em\) values in group 2 than in controls \((P < 0.05)\), but there was no change in the corresponding \(pD_2\) values (Fig. 1a; Table 1). The contractile responses to phenylephrine and both the \(Em\) and \(pD_2\) values in strips from rabbits in group 3 were not significantly different from those in group 2 (Fig. 1; Table 1). The contractions elicited by 124 mmol/L KCl were similar in all three groups of rabbits (Table 1).

Carbachol, adenosine and sodium nitroprusside produced concentration-dependent relaxation in pre-contracted strips in all three groups. Relaxation with carbachol was significantly greater in strips from castrated rabbits than in the controls \((P < 0.05)\); the concentration-response curve for carbachol was shifted to the left, with significantly higher \(Em\) values \((P < 0.05)\) (Fig. 1b; Table 1). The castration-induced changes in responsiveness to carbachol were significantly restored in the rabbits in group 3 (Fig. 1b). There were no significant changes in the \(pD_2\) values of strips from rabbits in group 2 or 3 compared with controls (Table 1).

Relaxation elicited by adenosine was significantly lower in castrated rabbits than in the controls \((P < 0.05)\). Despite the low maximum relaxation responses in group 2 to adenosine \((P < 0.05)\), the \(pD_2\) value calculated for adenosine-induced relaxation was not significantly different from that in the control group (Fig. 1c; Table 1). In group 3, the relaxant effects of adenosine were similar to those in the control group (Fig. 1c). The relaxation induced by sodium nitroprusside was similar in all groups of rabbits and there were no significant changes in the \(pD_2\) and \(Em\) values (Fig. 1d; Table 1).

In strips contracted by \(10^{-5}\) mol/L phenylephrine, EFS evoked frequency-dependent relaxation. Treatment with L-NAME decreased the electrically induced relaxation and increased tone in all strips (Fig. 2). In strips from group 2, the relaxant EFS effects remained unaltered at higher frequency \((32\,Hz)\) but at lower frequencies, EFS responses increased significantly \((P < 0.05)\;\text{Fig}\. 3)\. In strips from group 3, the relaxant effects of EFS were similar to those in the control (Fig. 3).

Discussion

Castrated rabbits showed a functional impairment of neurogenic and endothelium-dependent relaxation and phenylephrine-induced contraction in cavernosal tissue. Testosterone replacement for 1 month after castration had no significant effects on phenylephrine-induced contractile responses, while there were significant changes in both carbachol and EFS-induced relaxation responses. The precise mechanisms of penile erection are not yet fully understood, but the relaxation of cavernosal smooth muscle is critical in inducing and maintaining erection [9]. Corporeal smooth muscle relaxation and an increase in corporeal blood flow are necessary for penile tumescence and rigidity [10,21]. The mechanisms underlying the relaxation of penile smooth muscle are not fully understood, but both muscarinic receptor stimulation and release of relaxant nonadrenergic, noncholinergic (NANC) agents seem to be involved [22]. The endothelium and autonomic nerves are independent sources of NO, mediating cavernosal smooth muscle relaxation [23]. In vitro studies using EFS of rat, rabbit and human corpus cavernous tissue during muscarinic and adrenergic blockade have elicited NANC neurogenic relaxation. This effect was blocked by NOS inhibitors, indicating that NO is required for the response and supporting its role as the NANC neurotransmitter [9,11–13,20,24]. The complete blockade of electrically elicited relaxation of corporeal smooth muscle with the addition of tetrodotoxin (TTX) is consistent with the hypothesis that electrically elicited relaxation is mediated by the NANC pathway [13,20]. Some studies suggest that the cholinergic stimulation of muscarinic receptors on endothelial cells releases NO, creating a common pathway for cavernosal relaxation [25,26]. During detumescence, the smooth muscles of penile arteries and the sinusoids of the corpora cavernosa are contracted, thereby minimizing blood flow into the erectile tissue. This state of contraction is thought to be mediated mainly by the sympathetic nervous system stimulating post-junctional \(\alpha_1\)-adrenoceptors through the continuous release of noradrenaline into the synaptic cleft [14–16].

Many reports show that castration significantly attenuates sexual behaviour; such changes may be caused by effects of low plasma testosterone level on either the central nervous system or the peripheral neuro-cavernous mechanism [27,28]. The effects on the penis of low testosterone levels induced by castration are still unclear and controversial. The causes of organic erectile dysfunction in many men result from pharmacological abnormalities in the corporeal smooth muscle [10,16,18,21]. Some authors reported a loss of erectile response to either cavernosal nerve stimulation or to
Fig 1. Concentration-response curves of a. phenylephrine, b. carbachol, c. adenosine and, d. sodium nitroprusside (b–d were precontracted with $10^{-5}$ mol/L phenylephrine) in isolated strips of rabbit corpus cavernosum. a. Each point is expressed as a percentage of the contraction induced by 124 mmol/L KCl and shows the mean (SEM). b. c and d. Each point is expressed as a percentage of the contraction induced by $10^{-5}$ mol/L phenylephrine and shows the mean (SEM) *Significant differences from the control response. Numbers in parentheses indicate the number of preparations used. Dark green, Controls. Light green, Castration. Light red, Castration and testosterone.
Table 1 Maximum contractile response or relaxation (Em, in mg) and pD2 values on exposure to phenylephrine or agonists in strips of corpus cavernosum obtained from the three groups of rabbits

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Castrated</th>
<th>Castrated + testosterone</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Mean (sem)</td>
<td>n*</td>
<td>n</td>
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<tr>
<td>Phenylephrine</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Em</td>
<td>2100 (214)</td>
<td>8</td>
<td>1068 (108)††</td>
</tr>
<tr>
<td></td>
<td>5.46 (0.05)</td>
<td>8</td>
<td>1013 (103)††</td>
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<td>Potassium chloride</td>
<td></td>
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<tr>
<td>Em</td>
<td>1458 (293)</td>
<td>8</td>
<td>1396 (225)†</td>
</tr>
<tr>
<td></td>
<td>5.05</td>
<td>10</td>
<td>1430 (189)†</td>
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<tr>
<td>Carbachol</td>
<td></td>
<td></td>
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<tr>
<td>Em</td>
<td>1281 (386)</td>
<td>8</td>
<td>1830 (215)††</td>
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<tr>
<td></td>
<td>7.3 (0.02)</td>
<td>8</td>
<td>1464 (441)</td>
</tr>
<tr>
<td>Sodium nitroprusside</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Em</td>
<td>1441 (231)</td>
<td>8</td>
<td>1487 (238)</td>
</tr>
<tr>
<td></td>
<td>6.10 (0.01)</td>
<td>8</td>
<td>1409 (225)</td>
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<tr>
<td>Adenosine</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Em</td>
<td>850 (210)</td>
<td>8</td>
<td>375 (102)††</td>
</tr>
<tr>
<td></td>
<td>4.26 (0.01)</td>
<td>8</td>
<td>912 (226)</td>
</tr>
</tbody>
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*Number of observations. †P < 0.05, statistically different from control rabbits.

Fig. 2. The response to EFS in isolated strips of rabbit corpus cavernosum precontracted with phenylephrine (10⁻⁵ mol/L) and the inhibitive effect of L-NAME (3 × 10⁻⁵ mol/L) in the presence of guanethidine (5 µmol/L) and atropine (1 µmol/L).

acetylcholine injected intracavernosally in castrated animals. Takahashi et al. [6] found that castrated dogs showed a greatly diminished erectile response to acetylcholine injected intracavernosally but that the erectile response to nerve stimulation was similar to that in normal dogs. Baba [29] found that the relaxation response of cavernosal strips to acetylcholine and VIP decreased significantly in castrated animals but increased significantly in animals castrated but treated with testosterone replacement compared with controls. Thus, they suggested that testosterone plays an important role in erectile function by a direct action on the corpus cavernosum. Conversely, Anderson et al. [30] reported that castration enhanced NANC nerve-mediated relaxation in cavernosal tissue from rabbits. Possibly, the hormonal changes caused by castration may stimulate the synthesis and/or release of NO and/or suppress the release of noradrenaline from adrenergic nerves. However, Lin et al. [8] found that castration might not affect erectile ability; the slight reduction of maximal intracavernosal...
pressure in the castrated dog was attributable to decreased basal systemic blood pressure and not to effects on the penis. However, reports of the recovery of decreased sexual behaviour with sexual hormonal therapy are controversial, with some authors indicating that hormonal therapy in the castrated animal appears less effective on peripheral mechanisms and that the dependence of erectile response to testosterone is mediated centrally [8,31].

After testosterone therapy, mounting and intromission may be completely restored but ejaculation is not [28]. In isolated human corpus cavernosum pretreatment with testosterone had no effect on contraction or relaxation [32]. The present study shows that EFS of isolated precontracted strips of cavernosum from rabbit relaxed smooth muscle by mechanisms attributed to the formation and release of a relaxing factor with the properties of NO. The relaxation responses of EFS were decreased by the addition of L-NAME. This suggests that NANC neurotransmission is coupled in some way to the activation of the l-arginine-NO pathway in rabbit corpus cavernosum. These findings in rabbits parallel those in humans and Rajfer et al. [13] suggested that defects in this pathway may cause some forms of impotence. Supporting this, Ignarro et al. [11] found that electrically induced relaxation of the rabbit corpus cavernosum was associated with an increase in the cGMP content. This supports the hypothesis that stimulation of NANC neurotransmission in the corpus cavernosum triggers the endogenous formation of NO in NANC neurons, resulting in vascular smooth muscle relaxation [12]. In the present study, castrated rabbits showed an increased relaxation response to carbobal, but the relaxation response to nerve stimulation at higher frequency was similar to that in normal rabbits. Because the response to the NO donor sodium nitroprusside was the same in cavernosal tissue from controls and castrated rabbits, it may be assumed that the responsiveness of the erectile tissue to effects mediated by NO was unchanged. Testosterone is reported to have various effects on the density of some autonomic receptors [33] and therefore it is possible that the increased response to carbachol in castrated rabbits might be due to upregulation of muscarinic receptors on the endothelial cells of the sinusoidal spaces.

Adenosine is an endogenous physiological agent that has a potent inhibitory effect on a variety of neurophysiological phenomena [34]. The vasodilatory effect of adenosine on vascular beds is not mediated by endothelium-derived relaxing factor [35]. Adenosine increases cAMP levels by activating adenylate cyclase in smooth muscle cells [36]. Takahashi et al. [37] found that adenosine caused an increase in arterial flow and venous resistance, resulting in a full erection. In the present study, relaxation responses induced by adenosine in cavernosal strips were significantly lower after castration than in the controls. This may be a result of diminished purinergic receptor density.

In isolated preparations of human corpus cavernosum and spongiosum, and in penile arteries and veins, noradrenaline and phenylephrine produce concentration-dependent contractions [14–17]. a1-adrenoceptors predominate in human corpus cavernosal tissue and a1-adrenoceptors predominate in the cavernosal artery [17]. In some cases, impotence can be secondary to changes in α-adrenoceptor function [18,19]. Such in vitro observations support the supposition that alterations in corporal responsiveness to endogenous hormones and neurotransmitters may play a role in the cause of erectile dysfunction. Phenylephrine-induced contractility of rabbit corpus cavernosum decreased significantly in castrated animals compared with controls, but increased significantly in those given testosterone replacement [29], suggesting that testosterone plays an important role in erectile function. In the present study, phenylephrine-induced contractions were reduced in castrated rabbits when compared with controls; there were no differences in KCl-induced contractile responses among the groups. Thus, the contractile mechanisms were intact in the cavernosal smooth muscles. Hence, the decreased response to phenylephrine in castrated rabbits may be a steroid hormone effect of testosterone, which is known to directly affect the levels of neurotransmitter receptors. The decreased a1-adrenoceptor density after castration may be due to an associated downregulation of α-adrenoceptors. After castration, testosterone replacement for 1 month had no effect on phenylephrine-induced contraction.

In conclusion, the cavernosal tissue of castrated rabbits had a diminished contractile response to phenylephrine and an increased relaxation mediated by NO, which is known to be released from NANC nerves. These results clearly indicate that testosterone plays an important role in erectile function by a pre- or post-synaptic action on the corpus cavernosum.

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