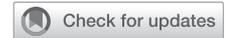


BASIC SCIENCE

Testosterone Induces Relaxation of Human Corpus Cavernosum Tissue of Patients With Erectile Dysfunction



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ABSTRACT

Introduction: Previous research in the field of cardiovascular diseases suggests a relaxing effect of testosterone (T) on smooth muscle cells. Therefore, it was hypothesized that T could play a significant role in erection development.

Aim: To investigate the relaxing effect of T and other molecules of the T signaling pathway on human corpus cavernosum (HCC) tissue.

Methods: Samples of the HCC tissue were obtained from men who underwent penile prosthesis implantation (n = 33) for erectile dysfunction. Samples were used for isometric tension measurement in Ex Vivo experiments. Following standardized precontraction with phenylephrine, increasing doses of T or dihydrotestosterone were administered and blocked by NO/H₂S synthesis inhibitors, a K_{ATP} blocker, and flutamide (androgen receptor inhibitor).

Main Outcome Measure: The outcome was relaxation of the HCC tissue, normalized to a maximum precontraction achieved by phenylephrine.

Results: A dose-dependent relaxing effect of dihydrotestosterone and T was observed with a relaxation of, respectively, 24.9% ± 23.4% (P < .0001) and 41.7% ± 19.1% (P = .01) compared with 6.8% ± 15.9% for vehicle (dimethylsulfoxide) at 300 μM. The relaxing effect of T was not countered by blocking NO synthesis, H₂S synthesis, K_{ATP} channels, or the androgen receptor.

Clinical Implications: By understanding the underlying mechanisms of T-induced HCC relaxation, potential new therapeutic targets can be identified.

Strengths & Limitations: The strength of the study is the use of fresh HCC tissues with reproducible results. The limitation is the need for supraphysiological T levels to induce the observed effect.

Conclusion: Rapid androgen-induced relaxation of HCC is likely to occur via nongenomic mechanisms. Previously suggested mechanisms of action by which T modulates HCC relaxation have been excluded. **Van den Broeck T, Soebadi MA, Falter A, et al. Testosterone Induces Relaxation of Human Corpus Cavernosum Tissue of Patients With Erectile Dysfunction. J Sex Med 2019; 8:114–119.**

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Key Words: Humans; In Vitro Techniques; Male; Muscle Relaxation; Muscle; Smooth/Physiology; Penis/Physiology; Testosterone/Physiology

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INTRODUCTION

Androgens are the main male sex steroids, responsible for the development and maintenance of the male phenotype during embryogenesis, puberty, and adulthood.¹ Testosterone (T) is the most abundant androgen in circulation ($\pm 90\%$), produced by Leydig cells in the testes² and is converted by the enzyme 5α -reductase to produce a more potent androgen, 5α -dihydrotestosterone (DHT).

The physiological actions of androgens are primarily mediated through the androgen receptor (AR),³ which is a ligand inducible transcription factor. Upon androgen binding, the AR binds specific sites in the genome, eventually resulting in differential gene transcription and protein synthesis.⁴ Within this classical genomic model, the androgen effects cannot occur sooner than the time it takes for the steroid to trigger gene transcription followed by protein synthesis, which typically peaks several hours after steroid exposure.⁵

Accumulating evidence, however, has shown that a nonclassical mode of androgen action exists, which at least initially is a nongenomic effect and is characterized by response times being seconds to minutes.

Multiple nongenomic modes of action have been described, with the most conserved cellular response to androgens being the rapid rise of intracellular calcium concentration, appearing within seconds to minutes upon androgen exposure.^{6,7} Specifically, androgens have shown to induce relaxation of the aorta and coronary arteries in a nongenomic fashion by interacting with the myocytes.^{6–13}

The erectile tissue of the penis consists of a ventral corpus spongiosum and 2 lateral corpora cavernosa, bordered by a dense collagenous tunica albuginea. The corpora contain irregular vascular spaces, lined by endothelium. Upon parasympathetic stimulation, the distributing arteries relax and the vascular spaces fill with blood. This leads to a distention of the corpora, pressing against the tunica albuginea, compressing the veins preventing blood to drain away. Therefore, for an erection to properly occur, adequate arterial relaxation is mandatory, which is one of the reasons (cardio)vascular diseases are an underlying cause of erectile dysfunction (ED). Based on the known effects of T on relaxation of coronary arteries, similar effects could be expected on the corporal arteries. Interestingly, comparing systemic and cavernous T levels, in healthy subjects, corporal T levels increased significantly compared with systemic T levels during erections. Based on these existing data, in this manuscript, we hypothesize that T and its derivatives play a role in the physiology of erection development and could potentially play a therapeutic role in patients with ED.¹⁴

METHODS

Study Population

Human corpus cavernosum (HCC) samples were obtained from 33 patients with ED of various etiologies during inflatable

penile prosthesis (IPP) implantation. All patients had normal preoperative sex hormone levels and were offered intracavernosal injections of prostaglandin E1 before considering IPP implantation. Patients consented to the use of penile tissues, and the study protocol was approved by the ethical review board at Jessa Hospital, Hasselt, Belgium, and the Biobank, Limburg, Hasselt, Belgium.

Isometric Tension Measurement Ex Vivo Studies

Immediately after harvesting during IPP implantation, tissue samples were immersed in ice-cold Krebs-Henseleit solution (NaCl 112 mmol/l, KCl 5.9 mmol/l, CaCl₂ 2.0 mmol/l, MgCl₂ 1.2 mmol/l, NaH₂PO₄ 1.2 mmol/l, NaHCO₃ 25 mmol/l, and glucose 11.5 mmol/l) and transported to the laboratory. The samples were divided into 6 equally sized longitudinal strips of $\sim 2 \times 5$ mm. These strips were then transferred into organ baths of 45 mL Krebs-Henseleit solution at 37°C and aerated with 95% O₂ and 5% CO₂. Strips were mounted between 2 clips and mounted to a force transducer. The height of the force transducer was adjustable to preload the mounted tissue strips. The force transducer was connected to PowerLab data acquisition system and LabChart recording software (ADInstruments, United Kingdom). The mounting was followed by a 60-min equilibration period, in which a preload tension was fixed at 1 g. Every 10 min, the tension was adjusted to maintain the preload of 1 g. After the equilibration period, the strips were precontracted with 10 μ mol/L phenylephrine. Ten minutes after precontraction, the baths were washed twice. This was repeated once more, after which the tissue was precontracted a third time with 10 μ mol/L phenylephrine. The maximum tension after this precontraction was defined as 100% tension. After stabilization of the tension traces, negative (dimethylsulfoxide [DMSO]) and positive (sodium nitroprusside [SNP] 10 μ mol/L) controls or the test compound was added. The investigated compounds were testosterone (Sigma-Aldrich, prod nr 86500), dihydrotestosterone (Sigma-Aldrich, prod nr 10300), flutamide (Flu) 350 nmol/L (AR inhibitor), DL-propargylglycine (PAG) 10 μ mol/L (H₂S synthesis inhibitor), β -cyano-alanine (BCA) 10 μ mol/L (H₂S synthesis inhibitor), glibenclamide 50 μ mol/L (K_{ATP} channel blocker), and N(ω)-nitro-L-arginine methyl ester (L-NAME) 500 μ mol/L (NO synthesis inhibitor). Subsequently, increasing concentrations of T or DHT were added (range, 0.3–300 μ mol/L).

Statistical Analysis

All values are expressed as mean \pm SEM. Statistical comparison between different compounds and their controls were performed using multiple t-testing when considering 1 compound and its respective control. When evaluating multiple compounds with their control and their potential interactions, two-way analysis of variance was performed. All statistical analyses were corrected for multiple testing. A *P*-value of $< .05$ was considered to indicate a statistically significant difference.

RESULTS

Patient characteristics and risk factors for ED are summarized in Table 1. The majority of the population had a history of smoking or was still an active smoker (67%), while only 9% of patients had type 2 diabetes. The median duration of ED was 6 (interquartile range, 3–10) years. Sex hormone levels were within normal range for all included patients (inclusion criterion) with a median total testosterone level of 4.6 (3.4–6.0) ng/ml; in 5 patients, the exact testosterone value was not available.

The relaxation effect of T and DHT on HCC was tested in organ bath experiments. The addition of increasing levels of T and DHT resulted in a dose-dependent relaxation of precontracted tissues. At a maximum concentration of 300 μ M T or DHT, $41.7 \pm 19.1\%$ ($P < .0001$) and $24.9 \pm 23.4\%$ ($P = .01$) relaxation was achieved, respectively, compared with $6.8 \pm 15.9\%$ for DMSO. At physiological plasma T levels of an adult human male (10–50 nM), no significant relaxing effect was observed (see Figure 1).

To investigate whether the observed relaxing effect of T was mediated through NO or H₂S signaling, the synthesis of NO and H₂S was inhibited during stimulation with T. The relaxing effect of T at 300 μ M (or any other concentration) was unaffected by the addition of L-NAME (NO synthesis inhibitor)

($P = .16$), PAG (H₂S synthesis inhibitor) ($P = .62$) and BCA (H₂S synthesis inhibitor) ($P = .49$) (Figure 2A–C). In addition, no effect was observed by adding the K⁺/ATP channel blocker Glibenclamide ($P = .84$) (Figure 2D).

Although it was unlikely – based on the short time to induce maximal relaxation by T – that the T-induced relaxation was mediated through its classical pathway by binding of the T-androgen receptor (AR) complex to DNA, this was confirmed by adding Flu to T which also did not influence the relaxing effect of T ($P = .25$) (see Figure 3).

DISCUSSION

The present study is one of the first to demonstrate rapid, dose-dependent relaxant responses of HCC to T and DHT based on ex vivo organ bath contractility experiments. Similar results were observed by Waldkirch et al.¹⁵ However, the patient population investigated in these experiments consisted largely of patients undergoing gender reassignment surgery, who are generally pretreated with hormonal therapies, which are known to influence corporal tissue composition.^{16–18} To ensure the validity of these findings in a more relevant clinical setting, only patients with IPP implantation for ED and normal preoperative sex hormone levels were included in this study.

Table 1. Descriptive characteristics of patients included in this study and subgroups for the different experimental setups

Characteristics	Total (N = 33)	T/DHT (n = 6)	L-NAME, PAG, BCA, Glibenclamide (n = 16)	Flutamide (n = 11)
Age at surgery (y), median (IQR)	61 (58, 66)	60 (57, 62)	63 (59, 65)	59 (57, 68)
Smoking, n (%)				
Current smoker	5 (15)	2 (33)	1 (6)	2 (18)
Previous smoker	17 (52)	3 (50)	10 (63)	4 (36)
Never smoked	7 (21)	1 (17)	4 (25)	2 (18)
Not reported	4 (12)	0 (0)	1 (6)	3 (27)
Type 2 diabetes, n (%)				
Yes	3 (9)	0 (0)	1 (6)	2 (18)
No	28 (85)	6 (100)	14 (88)	8 (73)
Not reported	2 (6)	0 (0)	1 (6)	1 (9)
Duration of ED (y), median (IQR)	6 (3, 10)	10 (3, 12)	6 (3, 10)	4 (3, 10)
Not reported, n (%)	5 (15)	1 (17)	1 (6.3)	3 (27)
Total Testosterone (ng/ml), median (IQR)	4.6 (3.4, 6.0)	5.5 (4.9, 6.5)	3.9 (3.2, 4.6)	6 (4.6, 6.6)
Not reported, n (%)	5 (15)	2 (33)	1 (6.2)	2 (18)
Doppler peak flow (cm/s), median (IQR)	10.7 (8.0, 13.1)	12.1 (10, 13.1)	9.4 (6.9, 13.6)	10.7 (9.9, 12.3)
Not reported, n (%)	6 (18)	0 (0)	2 (17)	4 (36)
Peyronie disease, n (%)				
Yes	5 (15)	1 (17)	2 (12.5)	1 (9)
No	28 (85)	5 (83)	14 (87.5)	10 (91)
Treatment for localized prostate cancer, n (%)				
Yes	6 (18)	0 (0)	4 (25)	2 (18)
No	26 (79)	6 (100)	12 (75)	8 (73)
Not reported	1 (3)	0 (0)	0 (0)	1 (9)

BCA = β -cyano-alanine; DHT = dihydrotestosterone; ED = erectile dysfunction; IQR = interquartile range; L-NAME = N(ω)-nitro-L-arginine methyl ester; PAG = DL-propargylglycine; T = testosterone.

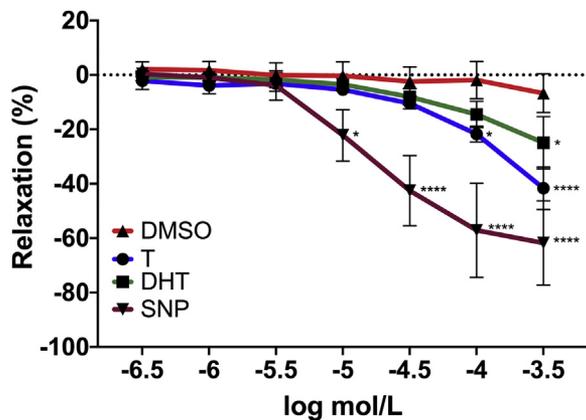


Figure 1. Isometric tension measurement assay of human corpus cavernosum tissue. The Y-axis represents the relative decrease in tension of the tissue after maximal contraction induced by phenylephrine. Data are normalized to maximal pre-contraction \pm SEM. DMSO and SNP, respectively, serve as the negative and positive control. T, testosterone; DHT, dihydrotestosterone; DMSO, dimethylsulfoxide; SNP, sodium nitroprusside; SEM, standard error of mean. Statistical analysis using two-way analysis of variance comparing the effect sizes of the reported drugs to DMSO. * $P < .05$, ** $P < .01$, *** $P < .001$, **** $P < .0001$.

The concentrations of T and DHT that were used to induce relaxation of HCC in this study were significantly higher than the circulating levels in adult human male plasma (10–50 nM). However, similarly, supraphysiological levels of T are necessary to induce vasorelaxation in aorta and the mesenteric artery in *in vitro* settings.^{12,19} In addition, in male patients with coronary heart disease, intravenous injection of supraphysiological doses of androgens significantly delays ST segment depression on electrocardiogram during exercise tests²⁰ and dramatically induces (brachial) artery vasodilatation, while the administration of physiological levels does not result in this effect.^{21,22} However, upon long-term administration of physiological T levels, vasodilatory effects are observed comparable with the acute effect at supraphysiological T levels.²³ Thus, evidence suggests that androgens applied within a physiological concentration range have a significant relaxant effect on vascular smooth musculature only after chronic exposure. The underlying cause for this phenomenon has not been elucidated yet. However, this could explain why in this experimental setup, high T levels are necessary to induce HCC tissue relaxation. Another potential reason for this observation could be inherent to the selected patient population. Changes in physiological function of the smooth muscle cells, extracellular matrix of HCC, and possibly tissue responsiveness to T are known to accompany patients suffering from ED.^{24,25}

Blocking NO and H₂S (2 neurotransmitters that have a well-described function in the development and maintenance of erections) synthesis by L-NAME, BCA, and PAG did not modulate the relaxation effect of T. Furthermore, although its role is still heavily debated, previous research has suggested that the vasodilating actions of T in the HCC tissue might occur through activation of K_{ATP} channels.²⁶ Interestingly, in this study, we could not replicate the results by Yildiz et al,²⁶ despite similar

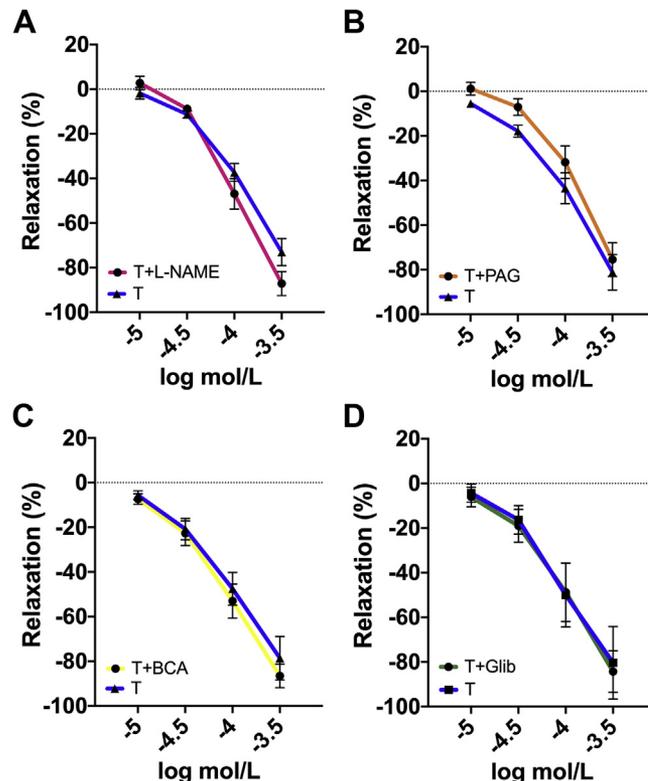


Figure 2. Isometric tension measurement assay of human corpus cavernosum tissue investigating the effect of (A) L-NAME, (B) PAG, (C) BCA, and (D) Glim on the relaxation induced by T. The Y-axis represents the relative decrease in tension of the tissue after maximal contraction induced by phenylephrine. Data are normalized to maximal pre-contraction \pm SEM. T, testosterone; L-NAME, N(ω)-nitro-L-arginine methyl ester; PAG, DL-propargylglycine; BCA, β -cyano-alanine; Glim; Glibenclamide; SEM, standard error of mean. Statistical analysis was performed using multiple t-testing. * $P < .05$, ** $P < .01$, *** $P < .001$, **** $P < .0001$.

experimental setup. However, in the latter study, denuded tissue samples were precontracted by KCl instead of phenylephrine (used in the presented study).²⁶ Previous research has already shown less T-induced relaxation of internal mammary arteries when precontracted with KCl (compared with prostaglandin 2 alpha), which can be explained by high extracellular K⁺ gradients altering activation of voltage-operated calcium channels.²⁷ This could also potentially alter sensitivity to further K⁺ channel (including K_{ATP}) blockage. Based on these findings, we question and add doubt to the role of K_{ATP} channels in T-induced relaxation in HCC tissues. Therefore, we conclude that its effects are (at least partially) mediated through other mechanisms.^{13,28}

Finally, in the classical genomic model, the effect of T is mediated through dimerization of the AR and its binding to DNA regulatory elements. However, because the observed relaxation effects of T in this study are observed within minutes, this is less likely, as the androgen effect in the genomic model typically only peaks several hours after steroid exposure.⁵ Furthermore, as the affinity to bind the AR is larger for DHT than that for T, it would be expected that the relaxing effect of

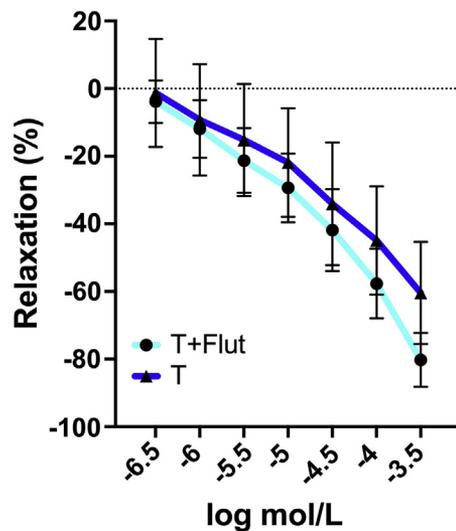


Figure 3. Isometric tension measurement assay of human corpus cavernosum tissue investigating the effect of flutamide on the relaxation induced by T. The Y-axis represents the relative decrease in tension of the tissue after maximal contraction induced by phenylephrine. Data are normalized to maximal pre-contraction \pm SEM. T, testosterone; Flut, flutamide; SEM, standard error of mean. Statistical analysis was performed using multiple t-testing. * $P < .05$, ** $P < .01$, *** $P < .001$, **** $P < .0001$.

DHT would be more pronounced than that of T. However, we have shown the opposite, suggesting that a nongenomic effect is at play. To exclude the role of the AR, AR dimerization was inhibited by the addition of Flu to T. Again, in these experiments, Flu did not modulate relaxation effects of T. As Flu switches from an AR antagonist to agonist, concentrations used in this study were within the antagonist range.²⁷ With Flu being a competitive AR-inhibitor, it cannot be completely excluded at very high concentrations, T could abolish the antagonistic effect of Flu. However, strongly supported by the existing literature in cardiovascular disease, it is very likely that the observed effects are mediated by a nongenomic effect of T.^{29,30} Furthermore, T-mediated vasodilation is also maintained in vessels isolated from testicular feminized mice, which lack a functional AR, further supporting that the AR is unnecessary for T-induced smooth muscle cell relaxation.²⁷

The strengths of this study are the use of rare, fresh HCC tissues and reproducible, dose-dependent observed effects. A limitation of the study is the need for supraphysiological T levels to induce the relaxation effect. Second, owing to ethical concerns, it was only possible to acquire the HCC tissue of men with ED and not of men with normal erectile function. Therefore, it remains unknown what the actual contribution of T is in HCC relaxation and erection development in a healthy in vivo setting.

CONCLUSIONS

Rapid androgen-induced relaxation of HCC is likely to occur via nongenomic mechanism in an ex vivo setting. We have excluded previously suggested mechanisms of action by which T

modulates HCC relaxation. Additional studies are required to further investigate the molecular mechanism causing T-induced HCC relaxation.

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