Korean J Physiol Pharmacol
Vol 12: 73－77, April, 2008

Testosterone Relaxes Rabbit Seminal Vesicle by Calcium Channel Inhibition

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Recent studies have documented that testosterone relaxes several smooth muscles by modulating K^+ channel activities. Smooth muscles of seminal vesicles play a fundamental role in ejaculation, which might involve testosterone. This study was aimed to assess the role of testosterone in seminal vesicular motility by studying its effects on contractile agents and on the ion channels of single vesicular myocytes in a rabbit model. The contractile responses of circular smooth muscle strips of rabbit seminal vesicles to norepinephrine (10 μM), a high concentration of KCl (70 mM), and testosterone (10 μM) were observed. Single vesicular myocytes of rabbit were isolated using proteolytic enzymes including collagenase and papain. Inside-out, attached, and whole-cell configurations were examined using the patch clamp technique. The applications of 10 μM norepinephrine or 70 mM KCl induced tonic contractions, and 10 μM testosterone (pharmacological concentration) evoked dose-dependent relaxations of these precontracted strips. Various K^+ channel blockers, such as tetraethylammonium (TEA; 10 mM), iberiotoxin (0.1 μM), 4-aminopyridine (4-AP, 10 μM), or glibenclamide (10 μM) rarely affected these relaxations. Single channel data (of inside-out and attached configurations) of BK channel activity were also hardly affected by testosterone (10 μM). On the other hand, however, testosterone reduced L-type Ca^{2+} currents significantly, and found to induce acute relaxation of seminal vesicular smooth muscle and this was mediated, at least in part, by Ca^{2+} current inhibition in rabbit.

Key Words: Testosterone, Seminal vesicle, Calcium channel, Ejaculation

INTRODUCTION

The seminal vesicles originate from the Wolffian duct under the influence of testosterone. After puberty, the glands form sac-like structures, which have a capacity of about 3.4～4.5 cm^2 and contribute to about 70% of the seminal fluid volume. Contraction of the muscular wall of seminal vesicle occurs under the influence of excitatory adrenergic and modulatory neuropeptide Y-encephalin-peptidergic nerve fibers (Aumuller & Riva, 1992). The seminal vesicles are androgen-responsive organs, and their structure and functions are regulated by androgenic steroids, which also inhibit the apoptosis and stimulate the proliferation of epithelial cells (Kashiwagi et al, 2005).

The seminal vesicles of gonadectomized rats also contract spontaneously, however, these contractions are of reduced amplitude, which could be related to atrophy and a reduction in smooth muscle cell numbers in seminal vesicles after castration (Hib et al, 1985). Androgens have potent effects on soma size and the transmitter levels of noradrenergic pelvic ganglion cells both before and after puberty (Melvin et al, 1988; Hamill & Schroeder, 1990). Seminal vesicular neurotransmitter systems include moieties, such as tyrosine hydroxylase, neuropeptide Y, and norepinephrine, and their expressions decrease after castration, although prevented by testosterone treatment (Hamill & Schroeder, 1990). Moreover, androgen-induced norepinephrine release from postganglionic neurons also mediates accessory sex organ smooth muscle proliferation (Kim et al, 2002).

If testosterone withdrawal lasts for long enough, seminal emission will eventually be impaired. Furthermore, testosterone replacement restores sexual interest and arousability, nevertheless, the majority of men have more circulating testosterone than is required to maintain a normal sexual function (Bancroft, 2005), suggesting that testosterone has important roles in addition to maintenance of sexual function. A number of studies have been conducted on the effects of androgen on the smooth muscle and autonomic nervous system of the reproductive tract. However, whether the observed effects are due to testosterone, its estrogen metabolites, or both is unknown, even in experimental animal models.

Testosterone could control parasympathetic sexual excitation in male, whereas sympathetic sexual excitation is modulated by a weak estrogenic stimulation (Motofei, 2001). Although it represents a minority of opinion, a few study found premature ejaculation to be associated with

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ABBREVIATIONS: TEA, tetraethylammonium; 4-AP, 4-aminopyridine; PSS, physiological salt solution; TES, testosterone.

hypogonadotrophic hypogonadism (Cohen, 1997). However, we found it hard to come up with an article concerning the relation between testosterone and ejaculatory disorders or on the direct effects of testosterone on normal seminal vesicular smooth muscle, although its androgenic action may be a matter of great importance during partial androgen deficiency or in the normal state.

The aim of this study was to assess the role of testosterone in the modulation of seminal vesicular motility by examining its effects on contractile agent induced-contraction and on the ion channels of single vesicular myocytes in a rabbit model.

METHODS

Animals and preparations

Twenty-one New Zealand White male rabbits (3 to 3.5 kg) were used in this study. All study protocols were approved by the ethical committee for the protection of persons and animals in biochemical research of the Institute of Medical Science, Chung Ang University, Seoul. Rabbits were anaesthetized with an overdose of pentobarbital (60 mg/kg, i.p.), immediately exsanguinated, and entire seminal vesicles were surgically removed. Excised tissues were immediately placed in 100% oxygen-saturated HEPES buffered physiological salt solution (PSS; composition in mM: NaCl 140; KCl 5; $CaCl_2$ 2; $MgCl_2$ 1; HEPES 10; D-glucose 11.5). Excised seminal vesicles were then carefully dissected from surrounding connective tissue. Briefly, isolated tissues were divided into strips of circular layer (approximately 2 mm diameter and 10 mm in length) for mechanical recordings and volumes of 1 mm^3 were digested for electrophysiological recordings. Tissue strips were placed in 15 ml organ chambers containing bicarbonate buffered PSS (composition in mM: NaCl 116; KCl 5; $CaCl_2$ 2; $MgCl_2$ 1; $NaHCO_3$ 24; D-glucose 11.5) and attached by a silk tie to a Model 52-9545 force transducer (Harvard, UK), and measurements were made on a MacLab 4e recording system (ADInstruments, Australia). Organ chambers were maintained at 37°C, and pH 7.4. Tissues were maintained at a 2 g resting tension, and strips were equilibrated for 2 hr with several changes of PSS.

Solutions and reagents

Bicarbonate buffered PSS was used for the organ bath studies and HEPES buffered PSS was used for the electrophysiological studies. To record whole cell Ca^{2+} channel currents, the external solution was changed to one containing $BaCl_2$ instead of $CaCl_2$ as a charge carrier. The solution contained 132 mM N-methyl-D-glucamine, 10 mM $BaCl_2$, 1mM $MgCl_2$, 5 mM $CsCl_2$, 10 mM HEPES, and 11.5 mM D-glucose, adjusted to pH 7.4 with 1 M CsOH, and the following solution was used as the internal (intrapipette) solution; 110 mM CsCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, 5 mM EGTA, 5 mM ATP, adjusted to pH 7.4 with 1 M CsOH. All chemicals were purchased from Sigma (USA).

Pharmacological responsiveness

To evaluate the effects of testosterone on contractile responses using strips of rabbit seminal vesicles, concentration-response curves were obtained for various contractile agents, such as acetylcholine and KCl. After strips had contracted to a steady state to the EC_{50} concentrations of these contractile agents, different doses of testosterone (10^{-9}~10^{-4} M) were added to tissues bath in a cumulative manner at 5 min intervals (Fig. 1). After the contractile response to each agent had stabilized, strips were washed with fresh PSS at least twice over 1 h and tensions were allowed to relax to the basal level.

Electrophysiological studies

The smooth muscles of rabbit seminal vesicle were washed and placed in Ca^{2+}-free HEPES buffered PSS. They were cut into 1 mm^3 cubes and placed in the same solution containing 0.1% bovine serum albumin, 0.1% collagenase and 0.1% trypsin inhibitor, and incubated for 30 min in a shaking water bath (37°C) to disperse the cells. They were then filtered and centrifuged, and the isolated smooth muscle cells obtained were stored in Ca^{2+}-free PSS at 4°C. Experiments were conducted at room temperature within 8 h of isolation. Conventional tight seal (seal resistance 5 to 10 GΩ) patch clamp methods were used to record currents on an Axopatch-1D patch clamp amplifier (Axon Instruments, USA). All analog signals were low pass filtered using an 8-pole Bessel type filter at 1 kHz and digitized at 2 kHz using pClamp 6 software (Axon Instruments, USA).

Statistical analysis

Data are expressed as means ± standard errors of mean (S.E.M.); n represents the number of strips or cells. All strip contractile responses are presented as developed tension (in newtons) divided by the strip wet weight (about 30 mg), and relaxation responses are presented as percentages of the maximal contractile responses elicited by the respective agents. EC_{50} values were calculated and obtained from regression lines, and each regression line was constructed using four to five points using the logistic sigmoid fitting model (Origin 6.0, USA). Student's t test and repeated measures analysis of variance were used for the analysis, and

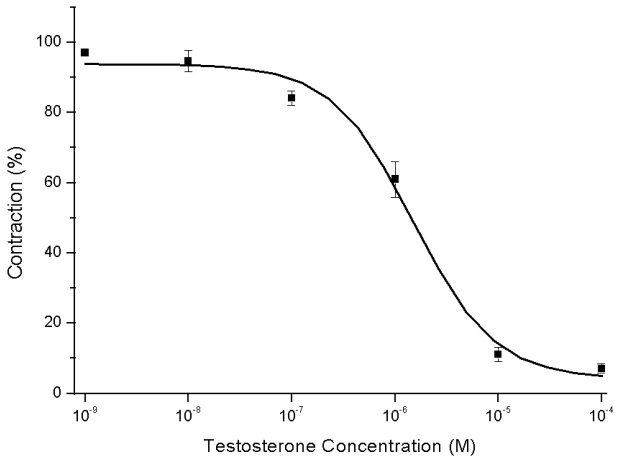

Fig. 1. Relaxation responses of circular smooth muscle strips of rabbit seminal vesicle. The IC_{50} was $1.49\times10^{-6}\pm4.43\times10^{-7}$ M (n=15).

p values of <0.05 were considered statistically significant.

RESULTS

Contractile responses induced by norepinephrine and KCl.

The contractile agents, norepinephrine and KCl, were used to evoke a sustained contractile status. These agents were applied at various effective concentrations; norepinephrine (10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} M), and KCl (35, 70, 105 and 140 M) and evoked dose-dependent sustained contractile responses (Fig. 2).

Effect of testosterone on norepinephrine- and KCl-induced contractions

Ten μM NE and 70 mM KCl were used to evoke a precontracted status. Norepinephrine-induced strip tension averaged 1.7±0.51 mN/mg and KCl-induced strip tension 0.87±0.28 mN/mg. Testosterone evoked powerful relaxations of both precontracted strips. And this response was hardly affected by actinomycin (10 μM) pretreatment (data not shown). The norepinephrine-induced strip tension average decreased to 0.13±0.082 mN/mg, and the KCl-induced average decreased to 0.062±0.0045 mN/mg (Fig. 3).

Involvement of K^+ channel in testosterone-induced relaxation

Following incubation with 1mM tetraethylammonium (TEA) for 30 min and then precontraction with KCl (70 mM), testosterone (10 μM) relaxed strip tension from 0.83±0.37 mN/mg to 0.064±0.0015 mN/mg, and after incubation with 10 mM TEA for 30 min and precontraction with KCl (70 mM), testosterone (TES; 10 μM) relaxed strips from 0.79±0.30 mN/mg to 0.081±0.0018 mN/mg (Fig. 4). Other K^+ channel blockers, including iberiotoxin (0.1 μM), 4-aminopyrine (10 μM), or glibenclamide (10 μM), also hardly affected testosterone-induced relaxation (data not shown).

Involvement of Ca^{2+} channel in testosterone-induced relaxation

Seven smooth muscle cells of rabbit seminal vesicle were

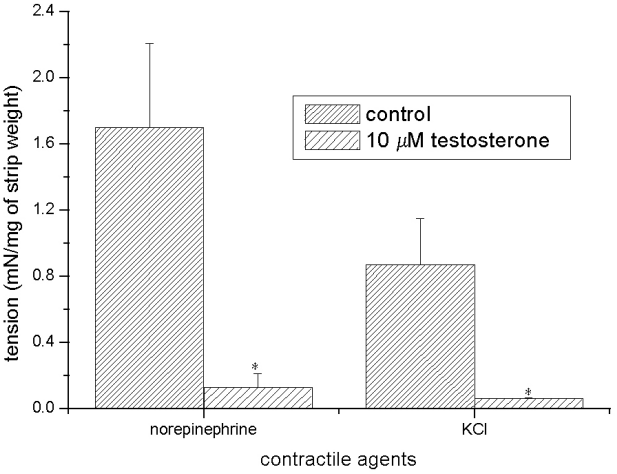

Fig. 3. Effect of testosterone on norepinephrine- and KCl-induced contractions. 10 μM norepinephrine and 70 mM KCl were used to evoke a precontracted status. 10 μM testosterone evoked powerful and significant relaxations of both precontracted strips (n=8). Asterisks indicate $p<0.05$ versus control group.

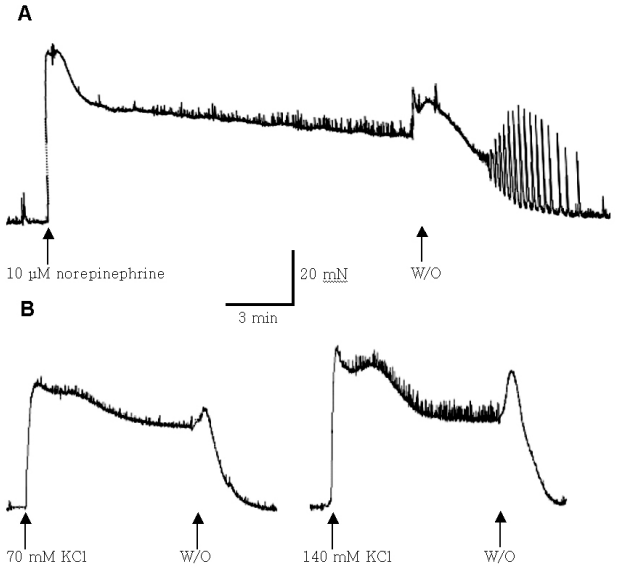

Fig. 2. Typical representations of contractile responses of circular smooth muscle strips of rabbit seminal vesicle. 10 μM norepinephrine (A) and high concentrations of KCl (70 and 140 mM; B) evoked sustained contractile responses. *W/O*, washout.

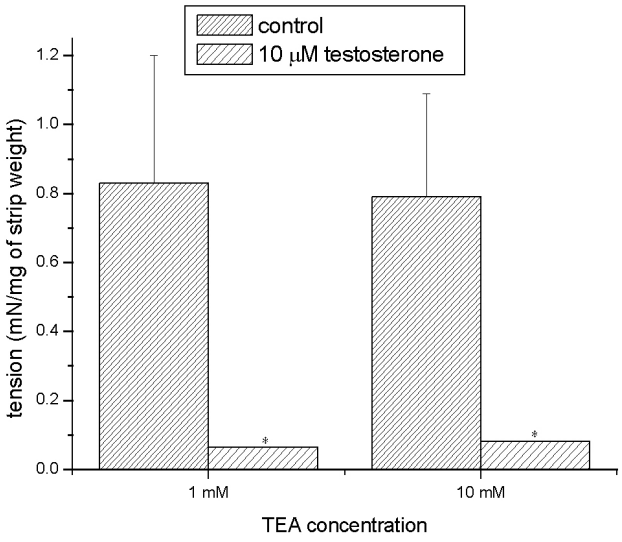

Fig. 4. Effect of TEA incubation on the testosterone-induced relaxation. Following incubation with 1 mM or 10 mM TEA and the strips were precontracted with 70 mM KCl, 10 μM TES still relaxed the strips (n=8). Asterisks indicate $p<0.05$ versus control group.

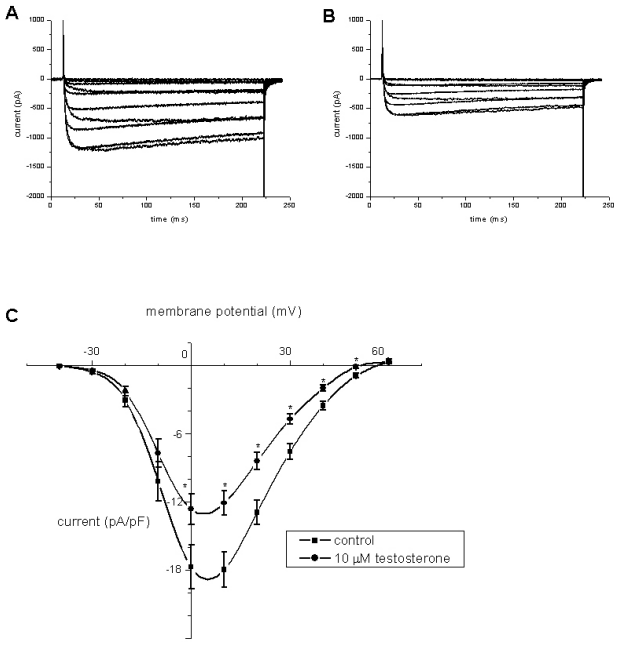

Fig. 5. Effect of 10 μM testosterone application on L-type Ca^{2+} channel currents. L-type Ca^{2+} channel currents appeared by depolarizing pulses from holding potentials of −50 mV (A). The currents were definitely decreased by 10 μM testosterone application (B). Effect of 10 μM testosterone on the current-voltage relations. (C) were obtained from a total of 7 smooth muscle cells of rabbit seminal vesicle. Depolarized conditions at above 0 mV testosterone significantly reduced the currents. Asterisks indicate $p<0.05$ versus control group.

used for L-type Ca^{2+} channel current recordings. These cells exhibited a mean resting potential, input resistance, membrane time constant, and cell capacitance of −42.4±3.2 mV, 12.5±2.1 GΩ, 64.3±9.7 msec and 15.2±1.3 pF, respectively. Whole cell Ca^{2+} channel currents and corresponding *I-V* relations were recorded from smooth muscle cells. The application of voltages to steps of 10 mV from a holding potential of −50 mV elicited an inward current at a threshold of about −10 mV. Testosterone (10 μM) significantly reduced inward currents, and this effect was observed at almost all voltages when Ca^{2+} current was activated (Fig. 5).

DISCUSSION

In the reproductive tract, testosterone promotes smooth muscle proliferation and enhances sympathetic neurotransmission. However, available testosterone levels normally exceed those required to maintain normal smooth muscular and neuronal structural integrity, and the role of this excess has not yet been elucidated. To our best knowledge, the present study is the first to describe the phenomenon of testosterone-induced relaxation of rabbit seminal vesicles in vitro.

Previous studies have demonstrated that testosterone relaxes rat thoracic aorta and rabbit coronary arteries in vitro and canine coronary arteries in vivo (Yue et al, 1995; Costarella et al, 1996). Moreover, the short-term administration of testosterone induces sex-independent vasodilation of coronary conductance and resistance arteries in vivo. And the acute testosterone-induced coronary vasodilation of epicardial and resistance vessels is known to be partly mediated by endothelium-derived NO (Chou et al, 1996). Testosterone has a direct vasodilating effect on rat aorta, which involves endothelium-independent mechanisms and is gender- and intracellular androgen receptor-independent (Costarella et al, 1996).

Androgen induced norepinephrine release from postganglionic neurons also mediates proliferation of accessory sex organ smooth muscles (Kim et al, 2002). The expressions of seminal vesicular tyrosine hydroxylase, neuropeptide Y, and norepinephrine decrease after castration, although these decreases can be prevented by testosterone treatment (Hamill & Schroeder, 1990). Guinea pig seminal vesicle smooth muscle displays an initial androgen-dependent proliferative response during early puberty, and this is followed by a progression to an androgen resistant amitotic state in adults (Kim et al, 2002). These results show that testosterone treatment may enhance sympathetic neurotransmission of seminal vesicles in the hypoandrogenic state and developmental period. In the present study, norepinephrine-induced seminal vesicular contraction was found to be inhibited by testosterone, suggesting that testosterone application within pharmacological dose may directly inhibit (1-adrenergic receptor-mediated contraction in seminal vesicle. Our present result supports the possibility that premature ejaculation is associated with testosterone inadequacy, as previously reported (Cohen, 1997). However, it should be noted that the present study was limited to in vitro experimentation and assessment of peripheral effect of testosterone on seminal vesicles.

Testosterone induces endothelium-independent relaxation in isolated rabbit coronary artery and aorta. Moreover, potassium channels, but not ATP-sensitive potassium channels, may be involved in the mechanism of testosterone-induced relaxation (Yue et al, 1995). Testosterone relaxes porcine coronary arteries predominantly by opening BK channels in coronary myocytes, and is involved in the activation of K^+ efflux through K^+ channels in vascular smooth muscle cells, perhaps via voltage-dependent (delayed-rectifier) K^+ channel (Deenadayalu et al, 2001; Ding & Stallone, 2001). Moreover, ATP-sensitive K^+ channels appear to play a role in the vasodilatory effect of testosterone on resistance arteries (Chou et al, 1996). In the present study, various potassium channel blockers (high TEA, iberiotoxin, 4-AP, or glibenclamide) failed to reverse testosterone-induced relaxation, and testosterone was found not to affect BK channel activity in seminal vesicular smooth muscle.

Androgens reduce increases of intracellular calcium concentrations in excited human myometrial smooth-muscle cells. Moreover, the blockade of L-type Ca^{2+} channels appears to be involved in the relaxing action of androgens (Perusquia et al, 2005), and testosterone directly inhibits vascular L-type Ca^{2+} channels in a voltage-independent manner at levels within the physiological range (Er et al, 2007). At higher (pharmacological) concentrations, T-type channels are also inhibited by testosterone (Scragg et al, 2004). In the present study, testosterone (at a pharmacological concentration) was found to relax the contractions induced by high K^+ and acetylcholine, through a mechanism possibly mediated by

the inhibition of L-type Ca^{2+} channel currents.

In conclusion, testosterone was found to induce acute relaxation of seminal vesicular smooth muscle, and this was found to be at least partly mediated by Ca^{2+} current inhibition in rabbit. Our findings suggest that testosterone may act as an inhibitory modulator of seminal vesicular contraction in the euandrogenic state.

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