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Mechanisms of vasorelaxation to testosterone in the rat aorta

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Abstract

We have investigated the role of endothelium-derived relaxing factors, K^+ channels and steroid receptors in vasorelaxation to testosterone in the rat aorta. Testosterone (1 nM–mM) caused acute concentration-dependent vasorelaxation. Both indomethacin (10 μM) and flurbiprofen (10 μM) uncovered relaxant responses to testosterone. The action of indomethacin was inhibited by endothelial removal. N^G-nitro-L-arginine methyl ester (L-NAME, 300 μM) had no effects on testosterone-induced responses. In the presence of indomethacin, the vasorelaxant potency of testosterone was reduced by depolarization with 60 mM KCl or charybdotoxin (100 nM), but not by glibenclamide (10 μM), 4-aminopyridine (1 mM) or barium chloride (30 μM). The responses to testosterone were not inhibited by flutamide (10 μM) or mifepristone (30 μM). Pre-treatment of the aorta with testosterone (100 μM) inhibited $CaCl_2$-induced contraction. In the present study, we have demonstrated that testosterone causes acute vasorelaxations, which are modulated via endothelium-derived prostanoids. The responses uncovered by cyclooxygenase inhibitors are due to the activation of K_{Ca} channels, while at higher concentrations, testosterone inhibits Ca^{2+} influx.

Keywords: Testosterone; K^+ channel; Vasorelaxation; Prostanoid; Ca^{2+} influx

1. Introduction

In men, the incidence of coronary heart diseases is much greater than in women of the same age (Adams et al., 1995; Wild and Bartholemew, 1988). This is reported to result from the unfavourable effects of the male sex hormone, testosterone (Adams et al., 1995; McCredie et al., 1998). For example, Adams et al. (1995) showed that testosterone increased progression of artheroma in hyperandrogenal female monkeys. In addition, testosterone also causes a decrease in high-density lipoprotein levels and an increase in low-density lipoprotein levels in both man and animals (Adams et al., 1995; McCredie et al., 1998; Wild and Bartholemew, 1988). In contrast, a correlative study by Glueck et al. (1993) carried out in hyperlipidemic men suggested that testosterone increased fibrinolytic activity and decreased fibrinogen, leading to a reduction of risk of coronary heart disease.

Several studies have shown that short-term administration of testosterone causes acute vasorelaxation in many vascular tissues from animals (Chou et al., 1996; Honda et al., 1999; Yue et al., 1995) and in man (Rosano et al., 1999; Webb et al., 1999). However, testosterone-induced vasorelaxation was previously only found at pharmacological concentrations (Chou et al., 1996; Costarella et al., 1996; Honda et al., 1999; Yue et al., 1995). In addition, the vascular mechanisms of testosterone-induced relaxation have not been clearly identified. Previous studies in rat aorta and coronary arteries from rabbit and canine have demonstrated that testosterone (1–300μM) causes acute endothelium-dependent vasorelaxations, which are likely to be mediated by activation of nitric oxide (NO) activity (Chou et al., 1996; Costarella et al., 1996; Honda et al., 1999). However, Yue et al. (1995) showed that a nitric oxide synthase (NOS) inhibitor had no effects on vasorelaxations to testosterone (100 nM–10 μM) in rabbit coronary and aorta. In addition, other studies reported that endothelium- and NO-independent vasorelaxations induced by testosterone (1–300 μM) may involve activation of K^+ channels in vascular smooth muscle cells (Chou et al., 1996; Honda et al., 1999; Yue et al., 1995).

We have recently demonstrated, in the rat mesenteric arterial bed, that testosterone causes acute vasorelaxation at physiologically relevant concentrations (100 pM–10 μM), which are, at least in part, mediated by endothelial NO and occur via steroid receptor-independent pathways. We also

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found that vasorelaxations induced by testosterone involve primarily activation of BK_{Ca} channels, as testosterone-induced responses were sensitive to tetrabutylammonium chloride and charybdotoxin (Tep-areenan et al., 2002a).

In the present study, we have now investigated the vascular mechanisms of testosterone-induced vasorelaxation in the rat aorta. The involvement of the endothelium and endothelium-derived relaxing factors, NO, prostanoids, endothelium-derived hyperpolarizing factor, and K^+ channels were first examined. Then, the Ca^{2+} channel antagonist effect of testosterone was also investigated. Finally, the involvement of steroid receptors in vasorelaxations induced by testosterone was also studied. Preliminary findings of this study have been published in abstract form (Tep-areenan et al., 2002b,c).

2. Methods

2.1. Preparation of the rat aorta

Male Wistar rats (250–300 g) were anaesthetized with sodium pentobarbitone (60 mg/kg, i.p.), and killed by cervical dislocation. Following a thoracotomy, the thoracic aorta was dissected from the rat. The aorta was cleaned of fat and connective tissue and cut into 5-mm ring segments. Each ring was transferred to a jacketed organ bath filled with 50 ml of Krebs–Henseleit solution (composition, mM: NaCl, 118; KCl, 4.7; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25; $CaCl_2$, 2; D-glucose, 10). The solution in the bath was maintained at a temperature of 37 °C and bubbled with 95% O_2 and 5% CO_2 mixture. The solution in the organ bath was exchanged every 15 min for 1 h. The rings were mounted between two triangular stainless steel hooks that were passed through the lumen and stretched to an optimal passive tension of about 1 g and maintained at this tension for 1 h. Tension was measured by an isometric force displacement transducer (LETICA 210), and recorded on a MacLab 4e recording system (AD instruments, New South Wales, Australia).

2.2. Experimental protocol of the rat aorta

Following a 1-h equilibration period, a high concentration of methoxamine (80–140 μM) was added to increase tension by approximately 0.50–1.00 g. In the presence of N^G-nitro-L-arginine methyl ester (L-NAME), a lower concentration of methoxamine (10–40 μM) was used to achieve equivalent tension as methoxamine became more potent. In some experiments, in order to investigate the possible involvement of K^+ channels in vasorelaxation to testosterone, 60 mM KCl was used to induce tension by substituting an equimolar concentration of NaCl with KCl (McCulloch et al., 1997). Once stable tension was established, various concentrations of testosterone (1 nM–1 mM) were added in a cumulative manner. Each inhibitor was added to the organ bath to establish the desired concentration and allowed a 1-h incubation period before cumulative concentrations of testosterone were added.

In vehicle-control experiments, ethanol alone was added cumulatively in the same volumes as those used in the experiments involving testosterone until the maximum concentration in the organ bath was 1.24% (v/v).

In order to investigate the role of the endothelium in responses to testosterone, the endothelium was removed by gently rubbing the luminal surface with a cocktail stick before mounting. The preparation was considered to be endothelium-denuded if vasorelaxation to 10 μM carbachol was less than 10%.

The involvement of prostanoids produced via the cyclooxygenase pathway in testosterone-induced vasorelaxation was assessed using the cyclooxygenase inhibitors, indomethacin (10 μM) and flurbiprofen (10 μM). An inhibitor of NOS, 300 μM N^G-nitro-L-arginine methyl ester (L-NAME, Randall and Griffith, 1991) was used to investigate the role of NO in vasorelaxation to testosterone. Indomethacin and L-NAME were also used in combination (see Table 1).

To investigate the involvement of K^+ channels (see Table 1) and a hyperpolarizing mechanism in vasorelaxation to testosterone, 60 mM KCl was used to induce tension (McCulloch et al., 1997) in the presence or absence of indomethacin, in combination with both indomethacin and L-NAME, or in endothelium-denuded preparations. In addition, 10 μM glibenclamide, a selective ATP-sensitive K^+ (K_{ATP}) channel inhibitor, 1 mM 4-aminopyridine, a voltage-sensitive K^+ (K_V) channel inhibitor, 30 μM barium chloride ($BaCl_2$), a voltage-dependent inward rectifier K^+ (K_{IR}) channel inhibitor (Andersson et al., 2000), or 100 nM charybdotoxin, an inhibitor of Ca^{2+}-activated K^+ (K_{Ca}) and K_V channels were also independently used to investigate the

Table 1

Agent	Pharmacological action	Reference
Indomethacin (10 μM) and flurbiprofen (10 μM)	Inhibition of cyclooxygenase	
N^G-nitro-L-arginine methyl ester (L-NAME, 300 μM)	Inhibition of nitric oxide synthase	Randall and Griffith (1991)
Glibenclamide (10 μM)	Inhibition of ATP-sensitive K^+ (K_{ATP}) channels	Randall and Griffith (1993)
4-aminopyridine (1 mM)	Inhibition of voltage-sensitive K^+ (K_V) channels	Honda et al. (1999)
Barium chloride ($BaCl_2$, 30 μM)	Inhibition of voltage-dependent inward rectifier K^+ (K_{IR}) channel	Andersson et al. (2000)
Charybdotoxin (100 nM)	Inhibition of an inhibitor of Ca^{2+}-activated K^+ (K_{Ca}) and K_V channels	Petersson et al. (1997)
Flutamide (10 μM) a	Testosterone receptor antagonist	Teoh et al. (2000)
Mifepristone (30 μM)	Steroid hormone receptor antagonist	Kalanic et al. (2000)

Summary of the pharmacological actions of the inhibitors used in this study.

types of K^+ channels that might be involved in vasorelaxations to testosterone.

The involvement of steroid receptors in vasorelaxation to testosterone was investigated using 10 μM flutamide, a testosterone receptor antagonist and 30 μM mifepristone, a steroid hormone receptor antagonist (see Table 1).

To investigate the Ca^{2+} channel antagonistic effect of testosterone, concentration–response curves to $CaCl_2$ (10 μM–30 mM) were obtained in the absence and in the presence of 100 μM testosterone. After aortic rings were allowed to equilibrate for 30 min, the rings were washed three times at 10-min intervals with Ca^{2+}-free Krebs solution (Salom et al., 2002). The rings were then bathed with Ca^{2+}-free, high KCl (100 mM) Krebs solution (Jiang et al., 1991) with or without testosterone (100 μM). In vehicle-control experiments, absolute ethanol was added in the same volume as 17β-oestradiol. After the rings were incubated with testosterone or ethanol for 30 min, the concentration–response curves to $CaCl_2$ (10 μM–30 mM) were constructed.

2.3. Data and statistical analysis

The concentration of vasorelaxant giving the half-maximal relaxation (EC_{50}) was obtained from the concentration–response curve. The data were best-fitted to one- or two-site models (i.e. with a high potency and a low potency); the most appropriate model was as determined by regression analysis and the r^2 values are stated. The EC_{50} values of curves fitted to a one-site model were determined by the logistic equation:

$$R = R_{\max} A^{n\mathrm{H}} / \mathrm{EC}_{50}^{n\mathrm{H}} + A^{n\mathrm{H}}$$

where R is the reduction in tone induced by methoxamine, A is the concentration of the relaxant, $R_{\max}$ is the maximum relaxation of the established tone, nH is the slope function and EC_{50} is the concentration of vasorelaxant giving half-maximal relaxation.

As appropriate, some data were fitted to a double hyperbola according to the following equation:

$$R = (R_{\max-1} A^{n\mathrm{H}-1} / \mathrm{EC}_{50-1}^{n\mathrm{H}-1} + A^{n\mathrm{H}-1}) + (R_{\max-2} A^{n\mathrm{H}-2} / \mathrm{EC}_{50-2}^{n\mathrm{H}-2} + A^{n\mathrm{H}-2})$$

where R is the reduction in tone, A is the concentration of the relaxant, $R_{\max-1}$ is the maximum relaxation of the established tone at the high potency site, $n\mathrm{H}-1$ is the slope function at the high potency site and EC_{50-1} is the concentration of vasorelaxant giving half-maximal relaxation at the high potency site. $R_{\max-2}$ is the maximum relaxation of the established tone at the low potency site, $n\mathrm{H}-2$ is the slope function at the low potency site and EC_{50-2} is the concentration of vasorelaxant giving half-maximal relaxation at the low potency site. The appropriateness of fitting the curves to one- or two-site model was determined by comparing their r^2 values.

Maximal responses are expressed as mean ± S.E.M., and pEC_{50} values (negative log of EC_{50} values) are expressed as means with 95% confidence intervals (CI). The number of animals in each group is represented by n. The data were compared, as appropriate, by the Student's unpaired t-test or analysis of variance (ANOVA) with statistically significant differences between groups being determined by Bonferroni's post hoc test. The curve-fitting and graph plotting were carried out using the graphical package GraphPad Prism.

2.4. Drugs and chemicals

All drugs and chemicals were purchased from Sigma (UK), except charybdotoxin, which was from Latoxan. Testosterone, indomethacin, flutamide and mifepristone were dissolved in absolute ethanol. Testosterone at a concentration of 100 mM was diluted to various concentrations in Krebs–Henseleit solution. Glibenclamide was dissolved in dimethyl sulphoxide. $BaCl_2$ and 4-aminopyridine were dissolved in distilled water. The remaining drugs were dissolved in the buffer. All drug solutions were prepared on the day of experiment.

3. Results

3.1. Vasorelaxation to testosterone in the rat aorta

In the rat aorta, testosterone (1 nM–1 mM) induced concentration-dependent vasorelaxation, which was rapid in onset, and the data were best-fitted ($r^2 = 0.999$) to a one-site model (control: $pEC_{50} = 4.39(4.25–4.54)$, with $R_{\max} = 168 \pm 5\%$, $n = 5$, Fig. 1). In vehicle-control experiments, ethanol caused a relaxation of 7.45 ± 1.17% ($n = 3$) at the maximal concentration used (1.24% (v/v), Fig. 1).

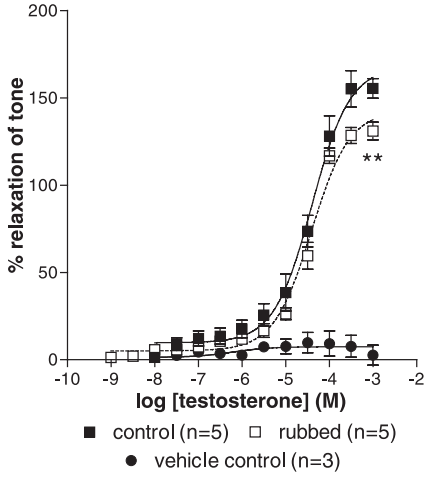

Fig. 1. Effects of removal of the endothelium on testosterone-induced vasorelaxation in the absence of indomethacin in the rat aorta. The relaxation is of methoxamine-induced tone. Data are shown as mean ± S.E.M. with vertical bars representing the S.E.M. **: $P < 0.01$ versus control.

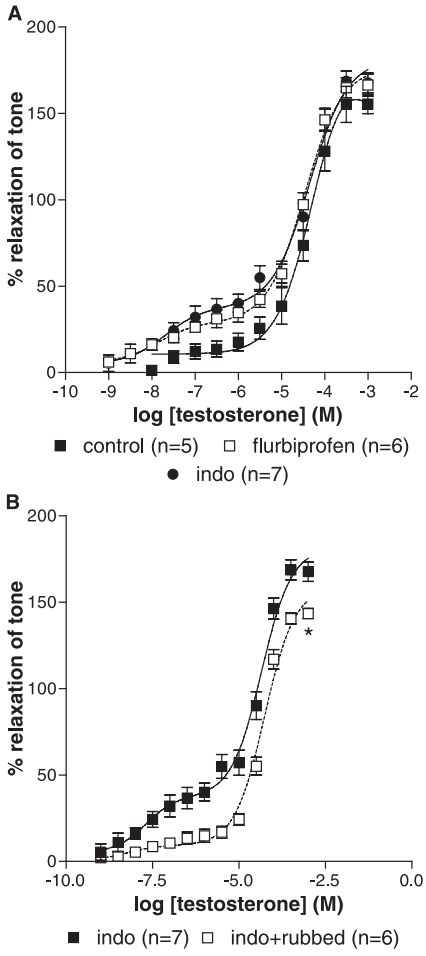

Fig. 2. (A) Effects of indomethacin (indo) and flurbiprofen on testosterone-induced vasorelaxation. (B) Effects of removal of the endothelium on testosterone-induced vasorelaxation in the presence of indomethacin in the rat aorta. In each case, the relaxation is of methoxamine-induced tone. Data are shown as mean ± S.E.M. with vertical bars representing the S.E.M. *: $P<0.05$ versus control.

3.2. Effects of endothelial denudation, cyclooxygenase inhibitors and L-NAME on testosterone-induced vasorelaxation

In the absence of indomethacin, removal of the endothelium produced a small but significant ($P<0.01$) decrease in the maximal relaxation to testosterone (control: $R_{max}=168\pm5\%$, $n=5$; rubbed: $R_{max}=142\pm5\%$, $n=5$), but had no effects on the potency of testosterone-induced vasorelaxation (control: $pEC_{50}=4.39(4.25–4.54)$, $n=5$; rubbed: $pEC_{50}=4.41(4.24–4.57)$, $n=5$, Fig. 1).

Pre-treatment with either indomethacin (10 μM) or flurbiprofen (10 μM) uncovered vasorelaxation at a high potency site and the data were now best-fitted ($r^2=0.999$) to a two-site model (indomethacin: $pEC_{50-1}=7.69(6.84–8.56)$, $pEC_{50-2}=4.35(4.14–4.55)$, $n=7$; flurbiprofen: $pEC_{50-1}=7.86(7.00–8.71)$, $pEC_{50-2}=4.47(4.34–4.59)$, $n=6$), but there were no changes in maximal relaxations to testosterone (control: $R_{max}=168\pm5\%$, $n=5$; indomethacin: $R_{max}=181\pm6\%$, $n=7$; flurbiprofen: $R_{max}=177\pm4\%$, $n=6$, Fig. 2A). In the presence of indomethacin, removal of the endothelium significantly inhibited maximal relaxation (indomethacin: $R_{max}=181\pm6\%$, $n=7$; indomethacin plus rubbed: $R_{max}=158\pm7\%$, $n=6$, $P<0.05$), and reduced the actions of testosterone at its high potency site of action such that the data were now best-fitted to a one-site model (indomethacin: $pEC_{50-1}=7.69(6.84–8.56)$, $pEC_{50-2}=4.35(4.14–4.55)$, $n=7$; indomethacin plus rubbed: $pEC_{50}=4.28(4.11–4.45)$, $n=6$, Fig. 2B).

Addition of both indomethacin (10 μM) and L-NAME (300 μM), or L-NAME alone had no effect on vasorelaxation to testosterone (L-NAME: $pEC_{50}=4.57(4.43–4.71)$, with $R_{max}=155\pm4\%$, $n=5$; indomethacin and L-NAME: $pEC_{50}=4.35(4.16–4.55)$, with $R_{max}=183\pm8\%$, $n=5$, Fig. 3).

3.3. Effects of high KCl and K^+ channel inhibitors on testosterone-induced vasorelaxation

In the absence of indomethacin, high extracellular K^+ had no effect on testosterone-induced vasorelaxation (control: $pEC_{50}=4.39(4.25–4.54)$, with $R_{max}=168\pm5\%$, $n=5$; KCl: $pEC_{50}=4.33(4.05–4.62)$, with $R_{max}=185\pm11\%$, $n=5$, Fig. 4A). Similarly, in endothelium-denuded rings, high extracellular K^+ did not affect vasorelaxation to testosterone in the absence of indomethacin ($pEC_{50}=4.29(4.08–4.51)$, with $R_{max}=165\pm8\%$, $n=5$, Fig. 4A).

In the presence of both indomethacin (10 μM) and L-NAME (300 μM), testosterone-induced vasorelaxation was not inhibited by high extracellular K^+ (indomethacin and L-NAME: $pEC_{50}=4.35(4.16–4.55)$, with $R_{max}=183\pm8\%$, $n=5$; indomethacin and L-NAME plus KCl: $pEC_{50}=4.23(4.06–4.41)$, with $R_{max}=206\pm9\%$, $n=5$, Fig. 4B).

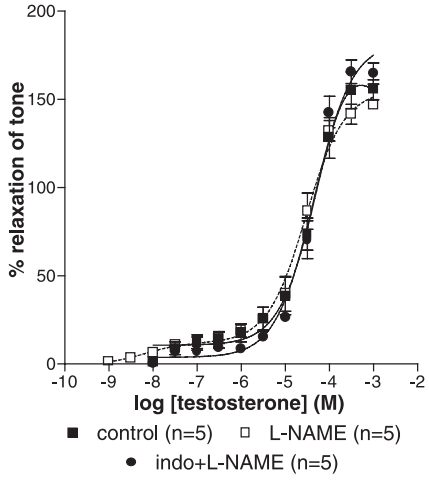

Fig. 3. Effects of N^G-nitro-L-arginine methyl ester (L-NAME, 300 μM), and addition of L-NAME and indomethacin (indo) on testosterone-induced vasorelaxation in the rat-isolated mesenteric arterial bed. The relaxation is of methoxamine-induced tone. Data are shown as mean ± S.E.M. with vertical bars representing the S.E.M.

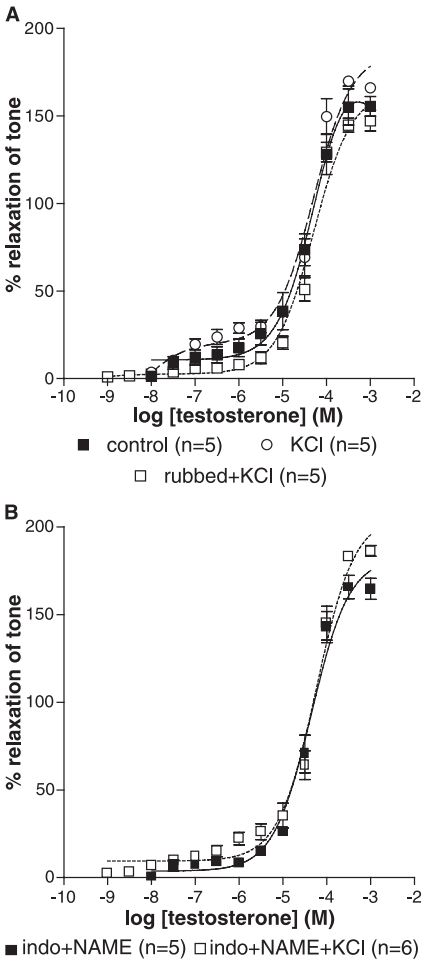

Fig. 4. (A) Effects of 60 mM KCl and removal of the endothelium on testosterone-induced vasorelaxation. (B) Effects of 60 mM KCl on testosterone-induced vasorelaxation in the presence of indomethacin (indo) and L-NAME in the rat-isolated mesenteric arterial bed. In each case, the relaxation is of induced tone. Data are shown as mean ± S.E.M. with vertical bars representing the S.E.M.

In the presence of indomethacin, high extracellular K^+ or pre-treatment with charybdotoxin (100 nM) abolished the actions of testosterone at its high potency site (indomethacin: $pEC_{50-1} = 7.69(6.84–8.56)$, $pEC_{50-2} = 4.35(4.14–4.55)$, $n = 7$; indomethacin + 60 mM KCl: $pEC_{50} = 4.19(3.98–4.40)$, $n = 7$; indomethacin plus charybdotoxin: $pEC_{50} = 4.39(4.20–4.57)$, $n = 4$), but had no effect on maximal relaxation (R_{max}: indomethacin = 181 ± 6%, $n = 7$; indomethacin plus KCl = 178 ± 10%, $n = 7$; indomethacin plus charybdotoxin = 184 ± 0%, $n = 4$, Fig. 5A).

Pre-treatment with 4-aminopyridine (1 mM), glibenclamide (10 μM), or $BaCl_2$ (30 μM) did not affect testosterone-induced vasorelaxation in the presence of indomethacin (indomethacin: $pEC_{50-1} = 7.69(6.84–8.56)$, $pEC_{50-2} = 4.35(4.14–4.55)$ with $R_{max} = 181 \pm 6\%$, $n = 7$; indomethacin + 4-aminopyridine: $pEC_{50-1} = 7.67(6.72–8.62)$, $pEC_{50-2} = 4.32(4.14–4.51)$ with $R_{max} = 183 \pm 6\%$, $n = 5$; indomethacin plus glibenclamide: $pEC_{50-1} = 7.76(7.05–8.47)$, $pEC_{50-2} = 4.34(4.22–4.45)$ with $R_{max} = 186 \pm 4\%$, $n = 5$; indomethacin + $BaCl_2$: $pEC_{50-1} = 7.99(6.95–9.03)$, $pEC_{50-2} = 4.44(4.33–4.55)$ with $R_{max} = 189 \pm 4\%$, $n = 9$; Fig. 5B).

3.4. Effects of steroid receptor antagonists on testosterone-induced vasorelaxation

Testosterone-induced vasorelaxation was not inhibited by pre-treatment with a testosterone receptor antagonist, flutamide (10 μM) in the absence of indomethacin (control: $pEC_{50} = 4.39(4.25–4.54)$, with $R_{max} = 168 \pm 5\%$, $n = 5$; flutamide: ($pEC_{50} = 4.32(4.05–4.59)$, with $R_{max} = 178 \pm 10\%$, $n = 5$, Fig. 6A). Similarly, in the presence of indomethacin, neither flutamide (10 μM) nor mifepristone (30 μM) reduced the potency or maximal relaxation to testosterone (indomethacin: $pEC_{50-1} = 7.69(6.84–8.56)$, $pEC_{50-2} = 4.35(4.14–$

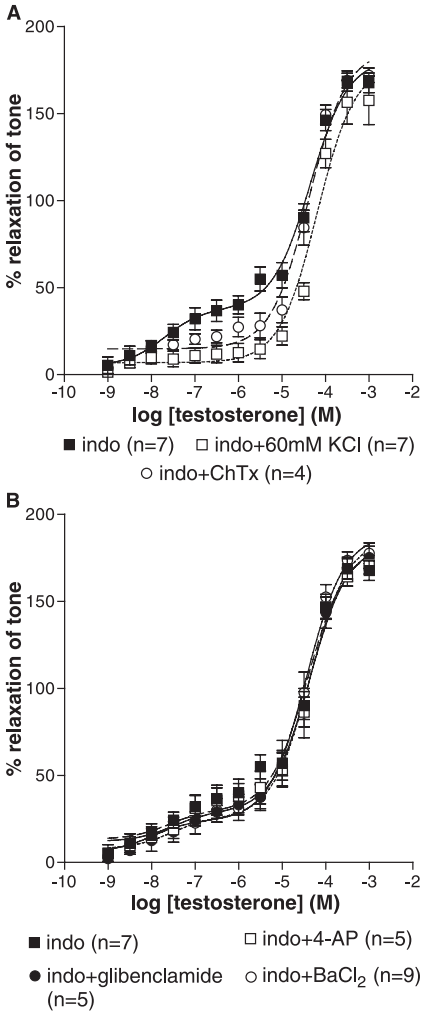

Fig. 5. (A) Effects of 60 mM KCl and charybdotoxin (ChTx, 100 nM) on testosterone-induced vasorelaxation in the presence of indomethacin (indo). (B) Effects of 4-aminopyridine (4-AP, 1 mM), glibenclamide (10 μM), and barium chloride ($BaCl_2$, 30 μM) on testosterone-induced vasorelaxation in the presence of indomethacin in the rat isolated mesenteric arterial bed. In each case, the relaxation is of induced tone. Data are shown as mean ± S.E.M. with vertical bars representing the S.E.M.

4.55) with $R_{max} = 181 \pm 6\%$, $n = 7$; indomethacin plus flutamide: $pEC_{50-1} = 7.70(7.09–8.32)$, $pEC_{50-2} = 4.48(4.26–4.69)$ with $R_{max} = 179 \pm 6\%$, $n = 6$; indomethacin plus mifepristone: $pEC_{50-1} = 7.69(7.46–8.46)$, $pEC_{50-2} = 4.49(4.27–4.71)$ with $R_{max} = 167 \pm 7\%$, $n = 6$). In contrast, the high potency portion of the testosterone-induced response was significantly ($P < 0.01$) enhanced by mifepristone, but not by flutamide (high potency portion (% of total relaxation response): indomethacin = 19 ± 3%, $n = 7$; indomethacin plus mifepristone = 27 ± 3%, $n = 6$; indomethacin plus flutamide = 34 ± 3%, $n = 6$, Fig. 6B).

3.5. Effects of testosterone on Ca^{2+}-induced contraction

In Ca^{2+}-free Krebs solution, 100 mM KCl increased tension by 0.09 ± 0.04 g ($n = 5$) above basal tension. Pretreatment with testosterone reduced tension by 0.30 ± 0.08 g ($n = 5$) below basal tension.

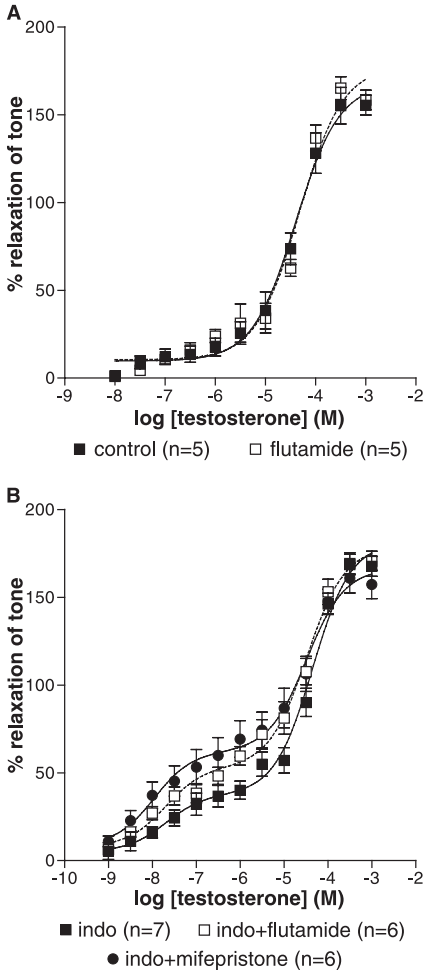

Fig. 6. (A) Effects of flutamide (10 μM) on testosterone-induced vasorelaxation in the absence of indomethacin. (B) Effects of flutamide (10 μM) and mifepristone (30 μM) on testosterone-induced vasorelaxation in the presence of indomethacin (indo). In each case, the relaxation is of methoxamine-induced tone. Data are shown as mean ± S.E.M. with vertical bars representing the S.E.M.

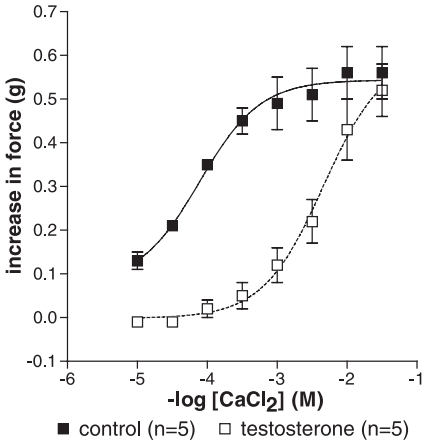

Fig. 7. Effects of testosterone (100 μM) on $CaCl_2$-induced contraction in male rat aortae depolarized by 100 mM KCl. Data are shown as mean ± S.E.M with vertical bars representing the S.E.M.

$CaCl_2$ (10 μM–30 mM) caused contraction in a concentration-dependent manner in the rat aorta depolarized by 100 mM KCl. Pre-incubation with 100 μM testosterone significantly ($P < 0.001$) inhibited $CaCl_2$-induced contraction at concentrations from 10 μM to 3 mM. However, testosterone did not affect contractions induced by 10 and 30 mM $CaCl_2$, Fig. 7.

4. Discussion

The present results have demonstrated in the rat aorta that testosterone (1 nM–1 mM) causes acute vasorelaxations, which are partly modulated via endothelium-dependent mechanisms and through inhibition of Ca^{2+} influx, but not through activation of steroid hormone receptors. Our results also show that endothelium-derived prostanoids may modulate the vascular responses to testosterone in the physiological concentration range. In addition, following blockade of the cyclooxygenase pathway, testosterone induces vasorelaxation by increasing K^+ efflux via K_{Ca}, but not through K_V, K_{ATP}, or K_{IR} channels.

We have recently reported that testosterone (100 pM–10 μM) causes acute and potent vasorelaxation in the rat mesenteric arterial bed pre-contracted with methoxamine (Tep-areenan et al., 2002a). In the present study, we also found in the rat aorta that testosterone (1 nM–1 mM) induced vasorelaxation in a concentration-dependent manner and had a rapid time of onset between 10 s and 5 min. Similarly, acute vasorelaxant effects of testosterone were previously reported in human (Rosano et al., 1999; Webb et al., 1999) and several other animal species (Chou et al., 1996; Yue et al., 1995). Given the rapidity of the actions, it is suggested that acute vasorelaxant effects of testosterone are mediated via non-genomic mechanisms. Interestingly, the present findings also showed that when the cyclooxygenase pathway was inhibited, testosterone could induce vasorelax-

ations in a physiological concentration range. These results are in agreement with our previous report in the rat mesenteric arterial bed showing that testosterone, at low concentrations (100 pM–1 nM), induces acute vasorelaxation, suggesting, therefore, that physiological (ca 20 nM; Johnson and Everitt, 1980) levels of testosterone may influence vascular tone. Furthermore, it should also be noted that the regulation of aortic vascular tone may have implications for aortic stiffness and pulse pressure in the intact cardiovascular system.

In the present study, we found that high concentrations of testosterone (100 μM–1 mM) caused vasorelaxation greater than 100% of established tone and this presumably reflects relaxation of myogenic tone.

Previous investigations have shown that testosterone-induced vasorelaxation was inhibited by removal of the endothelium in canine coronary artery (Chou et al., 1996) and aorta from spontaneous hypertensive rats (Honda et al., 1999). In the present study, we found that testosterone-induced vasorelaxation was only modestly inhibited by removal of the endothelium, ruling out a major role for endothelium-derived relaxants. Furthermore, the NOS inhibitor, L-NAME, did not affect testosterone-induced responses. Our findings in the rat aorta are consistent with the study by Yue et al. (1995) in rabbit coronary artery and aorta showing that neither L-NAME nor the guanylyl cyclase inhibitor, methylene blue, inhibited testosterone-induced vasorelaxation. In addition, previous studies also showed that testosterone had no effects on NOS activity in human and bovine aortic endothelial cells (Goetz et al., 1999; Hishikawa et al., 1995).

In the present study, pre-treatment with cyclooxygenase inhibitors, indomethacin and flurbiprofen uncovered vasorelaxation to testosterone at a high potency site. The action of the cyclooxygenase inhibitor was reduced by removal of the endothelium, indicating that testosterone-induced responses at the high potency site are masked by endothelium-derived prostanoids, such as thromboxane A_2. In contrast to our results, other studies have demonstrated that indomethacin did not affect testosterone-induced vasorelaxation in canine and rabbit coronary arteries, and rabbit aorta (Chou et al., 1996; Yue et al., 1995).

We then sought to investigate the role of K^+ channels in testosterone-induced vasorelaxation, as we have previously identified a major role for BK_{Ca} channels in the responses to testosterone in the rat mesenteric arterial bed (Tep-areenan et al., 2002a,b,c). In the rat aorta, high extracellular K^+ (60 mM KCl) did not inhibit vasorelaxation to testosterone in the absence of indomethacin. However, in the presence of indomethacin, high extracellular K^+ abolished testosterone-induced responses at its high potency site. Similar effects were also found after administration of charybdotoxin, an inhibitor of K_{Ca} and K_V channels. However, in the presence of indomethacin, pre-treatment with 4-aminopyridine, a K_V channel inhibitor, glibenclamide, a selective inhibitor of K_{ATP} channels or $BaCl_2$, a K_{IR} channel inhibitor, did not inhibit vasorelaxations to testosterone, ruling out the involvement of K_V, K_{ATP}, or K_{IR} channels in testosterone-induced responses. From these results, it is proposed that vasorelaxation to testosterone at the high potency site, which is modulated by endothelium-derived prostanoids, is mediated by increasing K^+ efflux through K_{Ca} channels. In agreement with our findings, Honda et al. (1999) showed that tetraethylammonium, a K_{Ca} channel inhibitor, inhibited testosterone-induced vasorelaxation in aortae from spontaneously hypertensive rats. A recent study has also demonstrated that vasorelaxation of porcine coronary artery to testosterone is inhibited by iberiotoxin, a highly selective BK_{Ca} channel inhibitor (Deenadayalu et al., 2001). In addition, a patch-clamp study by Deenadayalu et al. (2001) has shown that testosterone opens BK_{Ca} channels in porcine coronary smooth muscle cells. Testosterone-induced opening of BK_{Ca} channels is suggested to involve intracellular messengers (Deenadayalu et al., 2001; Ding and Stallone, 2001).

From the present findings showing that testosterone induced vasorelaxation, in part, via endothelium-dependent pathways and via activation of K^+ channels only when the cyclooxygenase pathway was inhibited, therefore, we then sought to investigate the inhibitory effects of testosterone on extracellular Ca^{2+} influx. We found that testosterone inhibited $CaCl_2$-induced contractions in the rat aorta depolarized by 100 mM KCl. These results indicated that testosterone inhibited contractile responses of the rat aorta to $CaCl_2$ by inhibiting Ca^{2+} influx from extracellular space. Our results are consistent with the previous findings showing that testosterone inhibits extracellular Ca^{2+} influx, but not Ca^{2+} release from intracellular stores in porcine coronary artery strips (Crews and Khalil, 1999a), rat aortic strips (Crews and Khalil, 1999b), and single coronary artery smooth muscle cells from pig (Murphy and Khalil, 1999). These results have been suggested that testosterone inhibited Ca^{2+} influx from extracellular space through voltage-gate Ca^{2+} channels (Crews and Khalil, 1999a), and/or via receptor-operated Ca^{2+} channels (Perusquía et al., 1996).

The present study also investigated the involvement of steroid hormone receptors in vasorelaxation to testosterone in the rat aorta. Our results showed that flutamide, a testosterone receptor antagonist, and mifepristone, a steroid receptor antagonist did not inhibit testosterone-induced vasorelaxation in the absence and in the presence of indomethacin. In fact, mifepristone and flutamide showed a tendency to enhance the high potency response, although the mechanism of this effect cannot be deduced from the data collected. These results indicate that, in the rat aorta, testosterone induces acute vasorelaxation via a steroid hormone receptor-independent pathway. Similar findings were also found in rabbit coronary artery and aorta that vasorelaxations to testosterone were not inhibited by flutamide (Yue et al., 1995). However, a recent study in porcine coronary smooth muscle cells demonstrated that testosterone-induced relaxation was sensitive to flutamide (Murphy and Khalil, 1999).

From these different findings, species and/or regional differences may involve differential distributions of steroid hormone receptors.

In summary, the present study in rat aortic rings has shown that testosterone induces acute vasorelaxations, which are mediated, at least in part, via inhibition of extracellular Ca^{2+} influx and modulated by endothelium-derived prostanoids. On inhibition of the cyclooxygenase pathway, testosterone causes vasorelaxations in physiological range and a high potency relaxant site is revealed which appears to involve activation of BK_{Ca} channels.

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