Testosterone-induced vasorelaxation in the rat mesenteric arterial bed is mediated predominantly *via* potassium channels

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1   We have investigated the involvement of nitric oxide and K^+ channels in the vasorelaxant responses to physiologically-relevant concentrations of testosterone in the rat isolated mesenteric arterial bed.

2   Testosterone (100 pM – 10 μM) elicited concentration-dependent relaxations in the isolated mesenteric arterial bed (pEC$_{50}$ = 9.47 (9.22 – 9.73, 95% CI), maximal relaxation, R_{max} = 62.8 $\pm$ 2.0%, n = 6). A nitric oxide synthase (NOS) inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME, 300 μM) or removal of the endothelium significantly inhibited maximal relaxations to testosterone (L-NAME: R_{max} = 51.4 $\pm$ 1.1%, P < 0.01, n = 6; endothelium-denuded: R_{max} = 46.9 $\pm$ 2.8%, P < 0.001, n = 5).

3   Raising the extracellular K^+ concentration to 30 and 60 mM, or pre-treatment with 300 μM tetrabutylammonium chloride (TBA), a calcium-activated K^+ channel inhibitor, abolished vasorelaxations induced by testosterone. A selective inhibitor of ATP-sensitive K^+ (K_{ATP}) channels, glibenclamide (10 μM) and an inhibitor of voltage-sensitive K^+ (K_V) channels, 4-aminopyridine (4-AP, 1 mM) did not affect testosterone-induced responses. Vasorelaxation to 1 μM testosterone was significantly (P < 0.05) inhibited by 100 nM charybdotoxin (ChTx), an inhibitor of large conductance calcium-activated K^+ (BK_{Ca}) channels (control: 63.3 $\pm$ 9.9%, n = 6; ChTx: 11.9 $\pm$ 12.7%, n = 3).

4   Neither the testosterone receptor antagonist, flutamide (10 μM) nor an aromatase inhibitor, aminoglutethimide (10 μM) inhibited testosterone-induced responses.

5   In conclusion, the present findings demonstrate, in the rat isolated mesenteric arterial bed, that testosterone causes acute vasorelaxations at physiologically relevant concentrations which are, in part, mediated *via* NO- and endothelium-dependent pathways. However, the activation of BK_{Ca} channels plays a substantial role in testosterone-induced vasorelaxation.
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Introduction

The incidence of cardiovascular diseases (CVD) and myocardial infarction is higher in men than women of a similar age (Wild & Bartholomew, 1988; Adams *et al.*, 1995). Indeed, testosterone is thought to be a risk factor for CVD and atherosclerosis (Wild *et al.*, 1988; Adams *et al.*, 1995; McCredie *et al.*, 1998). A previous study by Adams *et al.* (1995) of hyperandrogenism induced in female monkeys demonstrated that testosterone induced progression of atheroma and an increased level of serum low-density lipoprotein. Recently, McCredie *et al.* (1998) have shown that high-density lipoprotein was decreased in female to male transsexuals after long-term administration of androgen. However, Rosano *et al.* (1999) demonstrated that, in men with coronary artery disease, short-term administration of testosterone improved tolerance to exercise-induced myocardial ischaemia, perhaps suggesting that testosterone-induced

vasorelaxation may be involved in any cardiovascular protective effects of testosterone.

Several studies have shown that short-term administration of testosterone causes vasorelaxation in a range of species including human (Yue *et al.*, 1995; Chou *et al.*, 1996; Costarella *et al.*, 1996; Perusquia *et al.*, 1996; Crews & Khalil, 1999; Honda *et al.*, 1999). However, the mechanisms of testosterone-induced vasorelaxation are unclear. Costarella *et al.* (1996) demonstrated, in the rat thoracic aorta, that vasorelaxation to testosterone at pharmacological concentrations (25 – 300 μM) was inhibited by a nitric oxide synthase (NOS) inhibitor. Similar findings have also been reported in canine coronary arteries (Chou *et al.*, 1996). These results indicate that testosterone-induced vasorelaxation is mediated *via* activation of NOS activity (Chou *et al.*, 1996; Costarella *et al.*, 1996). Moreover, Honda *et al.* (1999) showed, in thoracic aorta from spontaneous hypertensive rats, that the vasorelaxation to testosterone is endothelium-dependent. In contrast, other studies have demonstrated that testosterone causes vasorelaxation in de-endothelialized rabbit coronary

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artery and rat thoracic aorta (Yue *et al.*, 1995; Costarella *et al.*, 1996; Perusquia *et al.*, 1996). Furthermore, Yue *et al.* (1995) have shown, in rabbit coronary artery and aorta, that vasorelaxation induced by testosterone was unaffected by a NOS inhibitor or methylene blue, an inhibitor of NO-activated guanylyl cyclase, and mediated *via* receptor-independent pathway.

The activation of K^+ channels on vascular smooth muscle cells is thought to be involved in the vasorelaxant effects of testosterone (Yue *et al.*, 1995; Chou *et al.*, 1996; Honda *et al.*, 1999). Yue *et al.* (1995) demonstrated, in the rabbit coronary artery, that vasorelaxation to testosterone (1 and 10 μM) was sensitive to barium chloride, a non-selective K^+ channel inhibitor, but not to glibenclamide, a selective inhibitor of K_{ATP} channels. Similarly, Chou *et al.* (1996) also showed in canine coronary arteries that vasorelaxation to testosterone (1 μM) was not affected by glibenclamide. In contrast, Honda *et al.* (1999) have recently demonstrated that vasorelaxation induced by testosterone at pharmacological concentrations was inhibited by glibenclamide in aortic rings from Wistar-Kyoto and spontaneously hypertensive rats.

In the context of vascular effects of sex hormones, we have recently shown that 17β-oestradiol (10 pM – 10 μM) causes acute and potent vasorelaxation, which were not mediated *via* NO. However, high extracellular K^+ inhibited vasorelaxation to 17β-oestradiol, suggesting, therefore, that 17β-oestradiol induces vasorelaxation by enhancing K^+ efflux. Furthermore, we found that 17β-oestradiol-induced responses were sensitive to TBA, an inhibitor of calcium-activated K^+ channels (Tep-areenan *et al.*, 2001).

On the basis of previous studies, the precise mechanisms of testosterone-induced vasorelaxation are still controversial. Therefore, the aim of the present investigation was to investigate the involvement of NO, the endothelium, K^+ channels, testosterone receptors, and aromatase in testosterone-induced vasorelaxation in the rat isolated mesenteric arterial bed.

Methods

Preparation of the rat isolated mesenteric arterial bed

Male Wistar rats (250 – 350 g) were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}, i.p.) and killed by cervical dislocation. The superior mesenteric artery was cannulated, and the arterial bed was dissected away from the guts and placed in a jacketed organ bath as previously described (McCulloch *et al.*, 1997). The arterial vasculature was perfused with oxygenated (95%O_2/5%CO_2) 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 plus 10 μM indomethacin) at a constant flow rate of 5 ml min^{-1} and maintained at 37°C. In the experiments involving high extracellular K^+, 30 mM and 60 mM KCl-containing isotonic Krebs-Henseleit solution was prepared by substituting an equimolar concentration of NaCl with KCl (McCulloch *et al.*, 1997).

Experimental protocol

The perfusion pressure in the superior mesenteric arterial bed was continuously monitored by a pressure transducer coupled to a Maclab 4e recording system (AD instruments, New South Wales, Australia). Flow rate was maintained at 5 ml min^{-1} and alterations in perfusion pressure represent changes in resistance of the mesenteric arterial bed.

Following a 30-min equilibration period, methoxamine (3 – 14 μM) was added to perfusion fluid to increase tone by approximately 70 – 100 mmHg. Once stable tone was established, testosterone was added cumulatively to the perfusion fluid (100 pM – 10 μM).

The involvement of NO in testosterone-induced vasorelaxation was assessed by using 300 μM N^G-nitro-L-arginine methyl ester (L-NAME), a NOS inhibitor (Randall & Griffith, 1991). In the presence of L-NAME, a low concentration of methoxamine (1 – 3 μM) was used to achieve equivalent tone, as the responses to methoxamine were enhanced.

In order to investigate the role of the endothelium in responses to testosterone, the endothelium was removed in some preparations by perfusion with distilled water for 10 min (Harris *et al.*, 2000). The preparation was deemed to be endothelium-denuded when the response to 55 nmol carbachol was less than 15%.

To examine the involvement of K^+ channels in vasorelaxation to testosterone, tone was established by KCl at concentrations of 30 mM (Yue *et al.*, 1995) and 60 mM (McCulloch *et al.*, 1997). The high extracellular K^+ concentration causes vasoconstriction by depolarization and inhibiting K^+ channel activity and thus preventing hyperpolarization and relaxation (Adeagbo & Triggle, 1993). In subsequent experiments, 300 μM tetrabutylammonium chloride (TBA), an inhibitor of calcium-activated K^+ channels (Randall & Kendall, 1997), 10 μM glibenclamide, an inhibitor of K_{ATP} channels (Randall & Griffith, 1993), and 1 mM 4-aminopyridine, an inhibitor of voltage-sensitive potassium (K_V) channels (Honda *et al.*, 1999) were independently used to investigate the types of K^+ channels involved in vasorelaxation to testosterone (10 pM – 10 μM).

To investigate the effects of a BK_{Ca} channel inhibitor, charybdotoxin (ChTx) at a concentration of 100 nM (Randall & Kendall, 1998), a test concentration of 1 μM testosterone was chosen and the effects of the toxin were examined against the response at this concentration.

The involvement of testosterone receptors and aromatase (to metabolize testosterone) in vasorelaxation to testosterone were examined by pre-treatment with 10 μM flutamide, a testosterone receptor antagonist (Teoh *et al.*, 2000), and 50 μM aminoglutethimide, an aromatase inhibitor (Yue *et al.*, 1995). In each case, the appropriate inhibitors were added to the perfusion fluid to establish the desired concentrations and allowed to equilibrate for 30 min. Then, testosterone was added in a cumulative fashion (100 pM – 10 μM).

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 1- or 2- site models; 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 1-site model were determined by the logistic equation:

$$R = R_{max} \bullet A^{nH}/EC_{50}{}^{nH} + A^{nH}$$

Where R is the reduction in tone, 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.

Maximal responses are expressed as mean $\pm$ s.e.mean, and pEC_{50} values (-ve 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 drawing were carried out using the graphical package GraphPad Prism.

Drugs and chemicals

All drugs and chemicals were purchased from Sigma Chemical Company (U.K.), except charybdotoxin, which was from Latoxan (France). Testosterone, flutamide, and indomethacin were dissolved in absolute ethanol. Testosterone, at a stock concentration of 10 mM in absolute ethanol, was diluted to working concentrations in Krebs-Henseleit solution; glibenclamide was dissolved in dimethyl sulphoxide; 4-aminopyridine was dissolved in distilled water; aminoglutethimide was dissolved in hydrochloric acid. The remaining drugs were dissolved in the perfusion fluid. All drugs were made upon the day of the experiment.

Results

Basal perfusion pressure and established tone

In the 60 experiments carried out, basal perfusion pressure was 15.4 ± 0.7 mmHg. Addition of methoxamine $(3-25 \ \mu M)$ increased perfusion pressure by 76.2 ± 4.2 mmHg $(n=22)$. In the presence of TBA, 4-AP, and aminoglutethimide, a higher concentration of methoxamine $(45-178 \ \mu M)$ was used to increase perfusion pressure by 67.0 ± 8.1 mmHg $(n=15)$. In the experiments involving glibenclamide, tone was increased by adding methoxamine $(45-100 \ \mu M)$ and 5-HT $(1.5-5 \ \mu M)$ to 88.0 ± 12.2 mmHg $(n=6)$, as methoxamine alone was inadequate. Addition of a NOS inhibitor, L-NAME $(300 \ \mu M)$ to the perfusion fluid did not affect basal perfusion pressure, but it was found necessary to use a lower concentration of methoxamine $(1-3 \ \mu M)$ to induce equivalent tone $(83.6 \pm 10.9$ mmHg, $n=5)$. Addition of 60 mM KCl to the Krebs-Henseleit solution increased the perfusion pressure by 75.2 ± 7.4 mmHg $(n=5)$ above basal level. After addition of 30 mM KCl to the perfusion solution, methoxamine was also added to induce tone $(96.1 \pm 2.1$ mmHg; $n=7)$, as 30 mM KCl alone was not enough to establish tone $(5.55 \pm 1.19$ mmHg).

Effects of L-NAME and endothelial denudation on testosterone-induced vasorelaxation

Testosterone $(100 \ pM - 10 \ \mu M)$ caused concentration-dependent relaxations of methoxamine-induced tone $(pEC_{50} = 9.47(9.22 - 9.73)$, 95% CI), maximal relaxation, $R_{max} = 62.8 \pm 2.0\%$, $n=6)$. L-NAME $(300 \ \mu M)$ significantly $(P < 0.01)$ reduced both the maximal relaxation to and potency of testosterone $(pEC_{50} = 8.79(8.65 - 8.95)$, $R_{max} = 51.4 \pm 1.1\%$, $n=6$; Figure 1). As shown in Figure 1, removal of the endothelium also significantly

$(P < 0.001)$ opposed maximal relaxation induced by testosterone $(R_{max} = 46.9 \pm 2.8\%$, $n=5)$ with no change in the potency $(pEC_{50} = 9.30(8.84 - 9.76)$, $n=5)$.

Effects of high extracellular K^+ concentrations and K^+ channel inhibitors on testosterone-induced vasorelaxation

Vasorelaxation to testosterone was significantly $(P < 0.001)$ inhibited by 30 mM KCl $(R_{max} = 9.12 \pm 4.86\%$, $n=7)$. Similarly, vasorelaxant effects of testosterone were abolished by 60 mM KCl and a contractile response was uncovered $(pEC_{50} = 7.66(7.23 - 8.10)$, $R_{max} = -19.8 \pm 2.4\%$, $P < 0.001$, $n=5$, Figure 2).

Testosterone-induced vasorelaxation was sensitive to TBA $(300 \ \mu M)$, and once again vasoconstriction was uncovered $(R_{max} = -25.3 \pm 5.9\%$, $P < 0.001$, $n=5$; Figure 3). Glibenclamide $(10 \ \mu M)$ and 4-AP $(1 \ mM)$ had no effect on testosterone-induced responses (glibenclamide: $pEC_{50} = 9.39(6.22 - 9.57)$, $R_{max} = 63.8 \pm 1.5\%$, $n=6$; 4-AP: $pEC_{50} = 8.89(8.48 - 9.31)$, $R_{max} = 86.6 \pm 3.7\%$, $n=5$, Figure 3).

The presence of ChTx $(100 \ nM)$ significantly $(P < 0.05)$ inhibited vasorelaxation to $1 \ \mu M$ testosterone (control: $63.3 \pm 9.9\%$, $n=6$; ChTx: $11.9 \pm 12.7\%$, $n=3$, Figure 4).

### Effects of testosterone receptor and aromatase inhibitors on testosterone-induced vasorelaxation

The addition of flutamide $(10 \ \mu M)$, a testosterone receptor antagonist did not inhibit testosterone-induced vasorelaxation, but significantly $(P < 0.001)$ enhanced maximal relaxation to testosterone $(pEC_{50} = 8.50(7.97 - 9.03)$, $R_{max} = 90.1 \pm 4.2\%$, $n=8)$.

Similarly, pre-treatment with aminoglutethimide $(10 \ \mu M)$, an aromatase inhibitor, did not oppose testosterone-induced responses, but significantly $(P < 0.01)$ augmented maximal relaxation to testosterone $(pEC_{50} = 8.27(7.83 - 8.70)$, $R_{max} = 73.8 \pm 3.2\%$, $n=5$, Figure 5).

Discussion

The results of the present study demonstrate that testosterone $(100 \ pM - 10 \ \mu M)$ causes potent and rapid vasorelaxations in the rat mesenteric arterial bed. The acute vasorelaxant effects
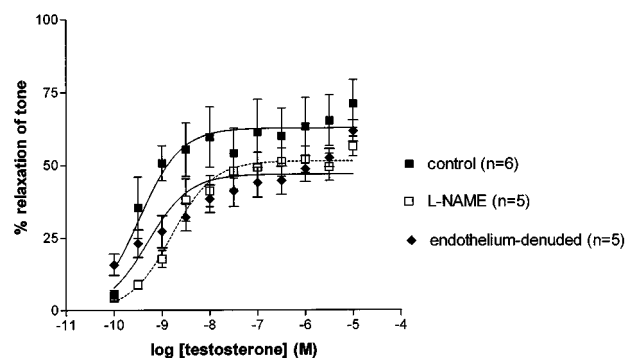

Figure 1 Effects of N^G-nitro-L-arginine methyl ester (L-NAME, $300 \ \mu M)$ and removal of the endothelium on testosterone-induced vasorelaxation in the rat isolated mesenteric arterial bed. Data are shown as mean $\pm$ s.e.mean.

of testosterone are partly mediated *via* endothelium- and NO-dependent, but not *via* receptor-dependent mechanisms. However, a substantial proportion of vasorelaxation to
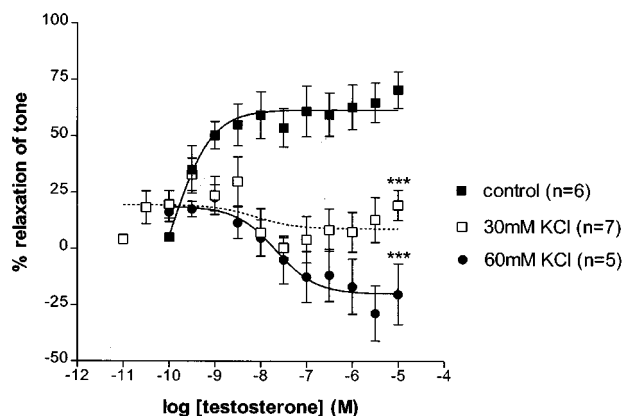
Figure 2 Effects of 30 and 60 mM KCl on testosterone-induced vasorelaxation in the rat isolated mesenteric arterial bed. Data are shown as mean±s.e.mean. ***Represents $P<0.001$ significantly different compared to control.
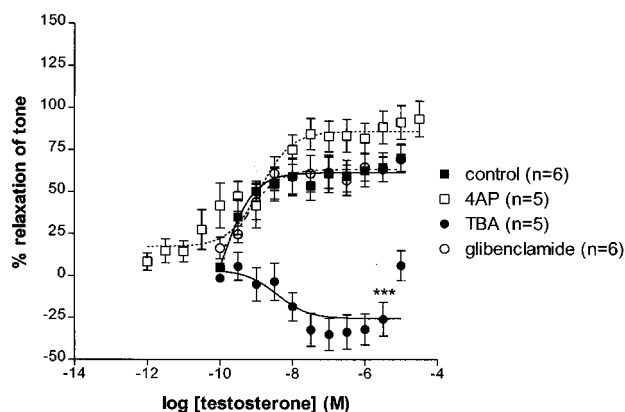
Figure 3 Effects of 4-aminopyridine (4-AP, 1 mM), tetrabutylammonium chloride (TBA, 300 μM), and glibenclamide (10 μM) on testosterone-induced vasorelaxation in the rat isolated mesenteric arterial bed. Data are shown as mean±s.e.mean. ***Represents $P<0.001$ significantly different compared to control.
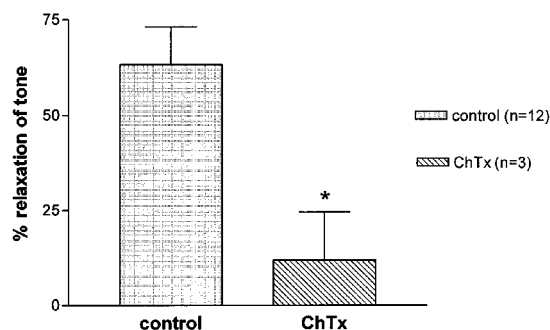

Figure 4 Effects of charybdotoxin (ChTx, 100 nM) on vasorelaxation to 1 μM testosterone in the rat isolated mesenteric arterial bed. Data are shown as mean±s.e.mean. *Represents $P<0.05$ significantly different compared to control.

testosterone is mediated by increasing potassium efflux through BK_{Ca} channels, but not *via* K_{ATP} or K_V channels.

In the present study, it was shown that testosterone caused acute vasorelaxations in the rat mesenteric arterial bed pre-contracted with methoxamine. These results are consistent with previous findings which show that testosterone induces vasorelaxation in contracted blood vessels, such as rat thoracic aorta (Costarella *et al.*, 1996; Perusquia *et al.*, 1996; Honda *et al.*, 1999) and porcine (Chou *et al.*, 1996), and human coronary arteries (Webb *et al.*, 1999). Several studies also demonstrated that acute vasorelaxant effects of testosterone were observed at pharmacological concentrations (100 nM – 300 μM) (Yue *et al.*, 1995; Chou *et al.*, 1996; Costarella *et al.*, 1996; Crews & Khalil, 1999; Honda *et al.*, 1999). Interestingly, the present findings showed that testosterone, at low concentrations (100 pM – 1 nM), induced acute vasorelaxation, suggesting, therefore, that physiological (ca 20 nM; Johnson & Everitt, 1980) levels of testosterone may influence vascular tone.

Crews & Khalil (1999) demonstrated, in rat aortic strips, that vasorelaxation to relatively high concentrations of testosterone (100 nM – 10 μM) had a rapid time of onset between 30 s and 2 min. Moreover, acute vascular effects of testosterone were also previously reported in an *in vivo* study by Chou *et al.* (1996) showing that testosterone (0.1 – 1 μM) caused coronary relaxation within 90 to 120 s. Our results confirm that relaxant responses of the rat mesenteric arterial bed to testosterone were rapid in onset, occurring within 30 s to 20 min. Therefore, it is probable that testosterone-induced vasorelaxation is due to non-genomic mechanisms.

In contrast to the present study, others have demonstrated that testosterone enhanced vasoconstrictor responses to various agents, such as $PGF_{2\alpha}$, potassium chloride, endothelin-1 and 5-HT (Farhat *et al.*, 1995; Teoh *et al.*, 2000), and attenuated the effects of the endothelium-dependent vasorelaxants, bradykinin and the calcium ionophore, A23187 (Teoh *et al.*, 2000). Clearly, in the present study testosterone caused relaxation rather than augmentation of methoxamine-induced tone. Although when K^+ channels were blocked vasorelaxations to testosterone were abolished in the presence of 60 mM KCl or TBA, and that, under these conditions, contractile responses to testosterone were uncovered. Therefore, it appears that testosterone can cause mesenteric vasoconstriction, but only when K^+ channels are inhibited.
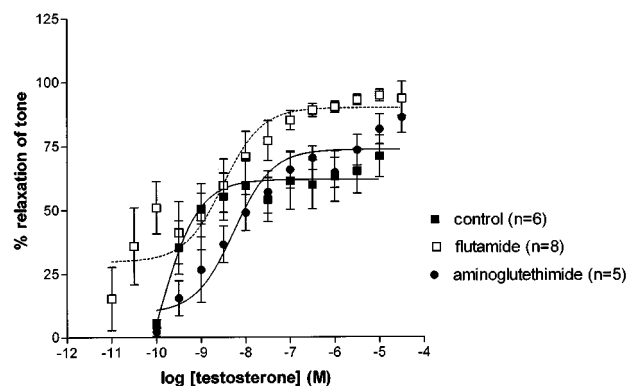
Figure 5 Effects of flutamide (10 μM) and aminoglutethimide (50 μM) on testosterone-induced vasorelaxation in the rat isolated mesenteric arterial bed. Data are shown as mean±s.e.mean.

Previous findings have demonstrated that vasorelaxation induced by testosterone was inhibited in the presence of NOS inhibitors in rat thoracic aorta (Costarella *et al.*, 1996) and canine coronary artery (Chou *et al.*, 1996). In the present investigation, we similarly found that testosterone-induced vasorelaxation was partly sensitive to L-NAME. Furthermore, we also found that removal of the endothelium opposed vasorelaxation to testosterone. These observations point to testosterone acting partly *via* endothelium- and NO-dependent pathways. Indeed, the effects of NOS inhibitor and removal of the endothelium had comparable effects, implying that NO is the principal endothelium-derived autacoid mediating these responses. The present findings are in agreement with the results of Chou *et al.* (1996), which showed that testosterone-induced vasorelaxation was inhibited in de-endothelialized canine coronary arteries. However, in our study there was a substantial endothelium-independent component. Indeed, other studies have demonstrated that neither removal of the endothelium nor the inclusion of NOS inhibitors affected vasorelaxation induced by testosterone in aortae from rat and rabbit, and coronary arteries from pig and rabbit (Yue *et al.*, 1995; Perusquia *et al.*, 1996; Crews & Khalil, 1999). Yue *et al.* (1995) also showed that methylene blue, an inhibitor of NO-activated guanylyl cyclase had no effects on testosterone-induced vasorelaxation in rabbit coronary artery and aorta. Furthermore, testosterone did not affect eNOS activity in the cultured bovine aortic endothelial cells (Goetz *et al.*, 1999).

Clearly endothelial-derived NO contributes modestly to testosterone-induced vasorelaxation, therefore, we then sought to investigate the role of K^+ channels. We found that vasorelaxation to testosterone was completely inhibited by raising the extracellular K^+ concentrations to 30 and 60 mM, indicating that testosterone acutely causes vasorelaxation mainly by increasing K^+ efflux (Adeagbo & Triggle, 1993). Furthermore, we also showed that testosterone-induced vasorelaxation was reduced by TBA, a selective inhibitor of calcium-activated K^+ channels and ChTx, a selective BK_{Ca} channel inhibitor, but not by glibenclamide, a K_{ATP} channel inhibitor or 4-AP, a voltage-sensitive K^+ channel inhibitor. From these results, we suggest that, in the rat mesenteric arterial bed, testosterone causes vasorelaxation *via* selective activation of BK_{Ca} channels. In agreement with our observations, Honda *et al.* (1999) recently demonstrated that tetraethylammonium, an inhibitor of calcium-activated potassium channels, inhibited vasorelaxation to testosterone in aortic rings from spontaneous hypertensive rats. In addition, previous studies also showed that glibenclamide had no effects on testosterone-induced vasorelaxation in coronary artery from rabbit (Yue *et al.*, 1995) and dog (Chou *et al.*, 1996). In contrast, neither glibenclamide or 4-AP inhibited vasorelaxation induced by

testosterone in aortic rings from both Wistar-Kyoto rats and spontaneous hypertensive rats (Honda *et al.*, 1999).

One possibility is that the endothelial-derived NO may act *via* activation of BK_{Ca} channels (Tare *et al.*, 1990). However, there was a substantial endothelium-dependent component of vasorelaxation to testosterone. This suggests that endothelial-derived NO cannot account for all of the BK_{Ca} channel activation, which must occur by some other mechanism.

The present findings showed that testosterone-induced vasorelaxation was not inhibited by a testosterone receptor antagonist, flutamide, but potentiated. From these results, it is indicated that vasorelaxation induced by testosterone is mediated *via* testosterone receptor-independent pathway. Our results are consistent with a previous study in rabbit coronary artery and aorta which showed that vasorelaxation to testosterone was unaffected by flutamide (Yue *et al.*, 1995). However, Murphy *et al.* (1999) demonstrated that testosterone inhibited contractile responses to prostaglandin $F_{2\alpha}$ and KCl in pig coronary smooth muscle cells, and this was sensitive to flutamide. This may point to species and/or regional differences in the involvement of testosterone receptors. The potentiated relaxation in the presence of flutamide might actually point to testosterone receptors being coupled to vasoconstriction. Blocking these receptors with flutamide might leave the non-receptor mediated vasorelaxation unopposed, leading to enhanced relaxation.

Based on the possibility that testosterone could be metabolized to oestradiol by an aromatase enzyme, we investigated the possibility that testosterone was metabolized to a vasoactive mediator by using an aromatase inhibitor, aminoglutethimide. Our results showed that aminoglutethimide had no inhibitory effects on testosterone-induced vasorelaxation. The present findings are in agreement with a previous study by Yue *et al.* (1995) which demonstrated that, in rabbit coronary artery, an aromatase inhibitor, aminoglutethimide had no effect on vasorelaxation induced by testosterone. In addition, Chou *et al.* (1996) also showed that pre-treatment with an oestrogen receptor antagonist, ICI 182,780 had no effects on vasorelaxation of canine coronary artery to testosterone (1 μM). Taken together, it is suggested that vasorelaxation to testosterone is unlikely to be due to vasorelaxant effects of oestrogen (Yue *et al.*, 1995; Chou *et al.*, 1996). The small potentiation due to aminoglutethimide might be explained by reduced local metabolism of testosterone.

In summary, our findings demonstrate that, in the rat mesenteric arterial bed, testosterone causes acute vasorelaxations at physiologically relevant concentrations, which are partly mediated by endothelial NO via receptor-independent pathways. The inhibitory effects of TBA and ChTx on testosterone-induced responses suggest that vasorelaxation to testosterone involves mainly BK_{Ca} channel activation.

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