Acute impairment of relaxation by low levels of testosterone in porcine coronary arteries

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Received 29 July 1999; accepted 27 October 1999  

Abstract  

Objectives: While there are many suggested reasons for the marked gender bias in cardiovascular events, much of the available data indicate that circulating estrogens are cardioprotective. The possibility that endogenous androgens may be detrimental to cardiovascular function has received relatively less attention. We investigated the short-term modulatory effects of various concentrations of testosterone on vascular function in isolated porcine coronary artery rings. Results: The higher concentrations (>1 μM) of testosterone relaxed U46619-contracted coronary artery rings in an endothelium-independent manner. This direct effect was insensitive to the testosterone receptor antagonists, flutamide and cyproterone acetate. Short-term exposure (20 min) to low levels of testosterone (1–100 nM), which were ineffective on their own on vascular function, significantly diminished relaxation to bradykinin and calcium ionophore A23187 but not those produced by levcromakalim and sodium nitroprusside. The inhibitory effect observed with 1 nM testosterone was only partially reversed by flutamide and cyproterone acetate and unaltered in the presence of actinomycin D and cycloheximide. Conclusions: These results demonstrate that acute treatment with testosterone, at concentrations that have no effect on their own, reduces vasorelaxation. Furthermore, they suggest that this modulatory action may be in part independent of the classical testosterone receptor since it was not completely sensitive to the anti-androgens and was not inhibited by the transcriptional and translational inhibitors. These findings support the postulation that testosterone may have unfavorable influences on vascular function. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Hormones; Receptors; Arteries; Vasodilation; Coronary circulation

1. Introduction  

Coronary artery disease (CAD) is a leading cause of mortality [1]. Epidemiological surveys indicate that premenopausal women demonstrate a lower incidence of CAD than do age-matched men [2]. However, this protection appears to fall rapidly following the onset of menopause [2]. Since long-term estrogen treatments improve vasodilatation [3,4] and reduce CAD morbidity and mortality [5,6], estrogens are considered to be cardioprotective. Interestingly, given the disparity of CAD risk between the sexes, the possibility of testosterone being detrimental to vasomotor function and hence predisposing males to CAD remains a relatively unexplored area. This is somewhat surprising considering the increasing clandestine use of androgenic-anabolic steroids and the association of premature cardiovascular events in young male athletes abusing such substances [7,8].

Currently, what minimal evidence there is on the influence(s) testosterone has on the vasculature is contradictory. Some indicate that testosterone levels are favorably linked with cardiovascular risk [9,10] and that plasma testosterone is higher in normotensive than in hypertensive men [11]. Hypoestrogenemia has been associated with CAD risk [12] and testosterone replacement...
ment appears to reverse this susceptibility [13]. Larsen et al. [14] found that chronic testosterone treatment did not promote atherogenesis in cholesterol-fed, castrated male rabbits. Conversely, testosterone administration paralleled endothelial dysfunction in hypercholesterolemic rabbits [15] and intensified atheroma in atherosclerotic female cynomolgus monkeys [16]. Furthermore, a causal relationship between chronic exposure to high amounts of male sex steroids and impaired endothelium-dependent vasodilation has been observed in female-to-male transsexuals [17]. Physiological levels of androgens also appear to be detrimental to the vasculature as adult men with a history of androgen deprivation therapy demonstrated better endothelium-dependent vasodilation than the age-matched control group [18]. However, confounding the issue further is the report by Bruck and colleagues [19] who found that while the atherogenic trend of chronic testosterone administration in female rabbits did not reach statistical significance, the same procedure exerted atheroprotective effects in male animals.

The mechanism(s) behind the acute actions of testosterone on the vascular system is still unclear as most of the literature has been derived from in vivo studies or in vitro experiments utilizing tissues of chronically treated animals. To the best of our knowledge, only two groups have investigated the acute vascular effects of testosterone using in vitro models [20,21]. However, they only addressed the influence of testosterone at high, micromolar concentrations. The current experiments were therefore designed to study the short-term direct and indirect influences of more physiological concentrations of testosterone on vasorelaxation in vitro.

2. Methods

2.1. Materials

U46619 (9,11-dideoxy-9α,11α-methanoepoxy prostaglandin F2α) was obtained from Biomol, Plymouth Meeting, PA, USA. Levromakalim was a gift from SmithKline Beecham, Harlow, Essex, UK. All other chemicals were from Sigma, St. Louis, MO, USA. Stocks of testosterone, flutamide, levromakalim and U46619 were made up in ethanol, A23187 in DMSO, cyproterone acetate in methanol and indomethacin in a buffered 1 mM sodium carbonate solution. The remaining drugs were dissolved in water. Working solutions were all obtained by serial dilutions in Krebs–Henseleit buffer.

2.2. Functional studies

Pigs were processed according to the regulations laid down by the Urban Services Department of the Government of Hong Kong. Porcine hearts of either sex were collected from the local abattoir and rinsed in cold, oxygenated (95% O2:5% CO2) Krebs–Henseleit solution (KHS; composition in mM: 120 NaCl, 4.76 KCl, 25 NaHCO3, 1.18 NaH2PO4·H2O, 1.25 CaCl2, 1.18 MgSO4·7H2O, 5.5 glucose) before the left anterior descending and right coronary arteries were isolated. After removal of connective tissue, coronary artery rings (3 mm) were suspended between stainless steel stirrups and stationary support rods positioned in 5 ml jacketed organ baths filled with oxygenated KHS maintained at 37°C. For experiments requiring endothelium-disrupted rings, porcine coronary arteries were halved. Each half was then perfused for 30 s with 0.5% Triton X-100 or KHS vehicle at a rate of 1 ml/min before being cut into 3 mm segments. All artery rings were placed under 2 g tension for at least 100 min before commencement of the experiment. Bath KHS was changed every 15 min during the whole equilibration period except for the last 30 min. Isometric tension was measured by force transducers (FT03, Grass Instrument Co., Quincy, USA) coupled to an amplifier and a personal computer for data collection (PICO Data Logger, Pico Technology Ltd., Cambridge, UK).

The viability of each porcine coronary artery ring was determined by pre-contracting with 30 nM U46619 (a thromboxane A2 analogue) to produce a sustained contraction. Ring samples were then relaxed with 1 μM bradykinin. Indomethacin (10 μM) was used to rule out the role of cyclooxygenase products such as prostacyclin from the preparation and this is commonly employed in most studies. Only rings that produced ≥4 g contraction and demonstrated ≥80% relaxation were used for further studies. In endothelium-disrupted preparations, rings which relaxed more than 5% were not used. After removal of these drugs by repeated washings, the rings were contracted under the same conditions as described above and the relaxational properties were measured by cumulative additions of the appropriate agents. In some experiments, testosterone (final concentration of 0.1–100 nM) was added to the baths 20 min prior to contraction with U46619. Where added, the steroid remained present throughout the experiment. Whenever the experimental protocol required the use of antagonists or transcription/translation inhibitors, these agents were added together at a concentration typical of similar in vitro studies (10 μM), and in the presence of indomethacin 20 min before the addition of testosterone. Preliminary findings indicated that responses of left anterior descending and right coronary arteries were not different, hence the results shown represent the pooled data from both artery types.

2.3. Data and statistical analyses

All data shown denote mean±SEM with n indicating the number of porcine hearts. Relaxations elicited by the various vasodilators were expressed as a percentage of U46619-induced contraction. Log EC50 values were determined according to the maximal response of each
individual response curve, with the aid of a curve-fitting program (SigmaPlot, Jandel Scientific Software, CA, USA). Analysis of variance (ANOVA) and Bonferroni’s test were applied where appropriate to determine individual differences between multiple groups of data using a computer statistical package (SPSS, SPSS Inc., Chicago, IL, USA). A P value of <0.05 was considered significant.

3. Results

3.1. Direct effect of testosterone on pre-contracted porcine coronary artery rings

Porcine coronary artery rings produced 5.74±0.22 g contractions to 30 nM U46619 (n=8). As illustrated in Fig. 1A, testosterone (0.1 nM–100 μM) relaxed U46619-pre-contracted rings in a concentration-dependent manner with the maximum response being 61.62±8.11% (n=8). Additions of the testosterone vehicle (final ethanol concentration in each bath ≤0.2%) instead of testosterone indicated that it did not influence the contractile efforts of U46619 significantly (Fig. 1A). The action of testosterone was not dependent on the presence of an intact endothelium since disruption of the endothelial layer did not significantly change the response measured (Fig. 1B). The testosterone receptor antagonists flutamide (10 μM) and cyproterone acetate (10 μM) proved to be ineffective against the direct relaxant effect of testosterone (Fig. 1C). Neither actinomycin D nor cycloheximide (both at final concentrations of 10 μM) managed to significantly influence this direct effect of testosterone (Fig. 1D).

Fig. 1. Direct relaxing effect of testosterone in porcine coronary artery rings. Endothelium-intact (A) and endothelium-damaged (B) ring segments were contracted with 30 nM U46619 and exposed cumulatively to vehicle (final bath ethanol concentration ≤0.2%, ●, n=8) or testosterone (○, n=8). (C, D) Each sample was incubated for 20 min with vehicle (●, n=8), 10 μM flutamide (○, n=8), 10 μM cyproterone acetate (△, n=7), 10 μM actinomycin D (○, n=8) or 10 μM cycloheximide (▲, n=8) before contracting with 30 nM U46619. Cumulative relaxation curves to testosterone (0.1 nM–100 μM) were then constructed. All data represent mean±SEM with n indicating the number of hearts investigated. * P<0.05 when compared to vehicle-treated rings (ANOVA with post-hoc Bonferroni’s test).
3.2. Effect of low concentrations of testosterone on endothelium-dependent and endothelium-independent relaxation in porcine coronary artery rings

Coronary artery rings were pre-contracted with 30 nM U46619 before constructing cumulative concentration-response curves to bradykinin (0.1 nM–1 μM), calcium ionophore A23187 (0.1 nM–1 μM), levcromakalim (1 nM–100 μM) and sodium nitroprusside (1 nM–100 μM). Under control conditions, rings contracted 5.47±0.11 g to 30 nM U46619 (n=28). As shown in Fig. 2, nanomolar concentrations of testosterone (1–100 nM), which on their own had no appreciable effect on relaxation, significantly shifted the relaxation-response curves of bradykinin and A23187 to the right and thus increased the EC_{50} values for these two relaxants significantly (Table 1). In addition, maximal responses to bradykinin, but not A23187 appeared to be reduced by exposure to testosterone (95.03±1.46% for vehicle-treated rings vs. 96.85±0.76, 86.94±0.88, 83.54±1.44 and 84.90±2.61% at 0.1, 1, 10 and 100 nM testosterone, respectively). This inhibitory action of testosterone was not completely concentration-dependent since alterations evoked by 10 and 100 nM testosterone were similar to that detected in the presence of 1 nM of the male steroid while 0.1 nM testosterone did not appreciably affect the bradykinin- and A23187-response curves (Fig. 2). In contrast to the effects observed with bradykinin and A23187, the levcromakalim and sodium nitroprusside relaxation curves were unaffected by all four concentrations (0.1, 1, 10 and 100 nM) of testosterone studied (Fig. 3, Table 1). In order to examine if a low level of testosterone impairs endothelium-dependent relaxation in a time-dependent manner, some rings were incubated with testosterone for 4 h instead of 20 min. As shown in Fig. 4, this impairment of relaxational responses to bradykinin and A23187 was conserved, even after a longer 4 h exposure to testosterone.

3.3. Effect of anti-androgens on the inhibitory action of testosterone on endothelium-dependent relaxation

In order to elucidate the possible participation of the classical testosterone receptor in the observed inhibitory response, some preparations were treated with recognized testosterone receptor antagonists. Neither flutamide nor cyproterone acetate (both at a final bath concentration of 10 μM) were able to completely reverse the modulatory action of 1 nM testosterone on bradykinin- and A23187-induced relaxation (Fig. 5). Table 2 shows the EC_{50} values calculated for bradykinin and A23187 in the absence and presence of testosterone, flutamide and cyproterone acetate.

3.4. Role of transcription and protein synthesis in the inhibitory action of testosterone on endothelium-dependent relaxation

Actinomycin D (10 μM) or cycloheximide (10 μM) were included in the incubation protocol for some experiments. As shown in Fig. 6 and Table 3, the attenuating action of 1 nM testosterone was insensitive to both of these agents regardless of the relaxing agent applied to the ring preparation.  

4. Discussion

We have found that short-term exposure (20 min) of porcine coronary artery rings to low concentrations (1–100
Table 1
Log EC_{50} values of bradykinin, A23187, levocromakalim and sodium nitroprusside in U46619-contracted porcine coronary artery rings following 20 min incubation with ethanol vehicle or testosterone (0.1–100 nM)^a

<table>
<thead>
<tr>
<th>Relaxing agent</th>
<th>n</th>
<th>Vehicle</th>
<th>0.1 nM Testosterone</th>
<th>1 nM Testosterone</th>
<th>10 nM Testosterone</th>
<th>100 nM Testosterone</th>
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<td>Bradykinin</td>
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<td>7.99±0.01</td>
<td>7.86±0.04*</td>
<td>7.83±0.04*</td>
<td>7.75±0.04*</td>
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<tr>
<td></td>
<td></td>
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<td>[10.2]</td>
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<td>[17.6]</td>
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<td>A23187</td>
<td>7</td>
<td>7.26±0.02</td>
<td>7.27±0.02</td>
<td>7.03±0.04*</td>
<td>6.96±0.02*</td>
<td>6.92±0.04*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[55.5]</td>
<td>[53.7]</td>
<td>[94.2]</td>
<td>[109.4]</td>
<td>[121.6]</td>
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<tr>
<td>Levocromakalim</td>
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<td>6.54±0.07</td>
<td>6.54±0.02</td>
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<td>6.51±0.01</td>
<td>6.53±0.01</td>
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<tr>
<td></td>
<td></td>
<td>[289.1]</td>
<td>[288.4]</td>
<td>[287.1]</td>
<td>[311.2]</td>
<td>[295.1]</td>
</tr>
<tr>
<td>Sodium nitroprusside</td>
<td>8</td>
<td>5.96±0.05</td>
<td>5.99±0.02</td>
<td>6.03±0.04</td>
<td>5.91±0.09</td>
<td>5.97±0.05</td>
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<td></td>
<td>[1086.4]</td>
<td>[1023.3]</td>
<td>[935.1]</td>
<td>[1219.0]</td>
<td>[1071.5]</td>
</tr>
</tbody>
</table>

^a Data represent mean±SEM with n indicating the number of hearts investigated. Log EC_{50} values are calculated from concentration-response relationships illustrated in Figs. 2 and 3. Mean EC_{50} values are denoted in square brackets. Statistical analysis was performed using ANOVA followed by Bonferroni’s test. *P<0.05 compared with corresponding vehicle control value. **P<0.05 compared with corresponding value for rings treated with 0.1 nM testosterone.

Fig. 3. Influence of testosterone on the relaxation responses of porcine coronary artery rings to (A) levocromakalim and (B) sodium nitroprusside (SNP). Each ring was treated for 20 min with vehicle ( ), 0.1 nM (○), 1 nM (△), 10 nM (▲) or 100 nM (▼) testosterone before being contracted with 30 nM U46619 and cumulatively relaxed with either levocromakalim or sodium nitroprusside. Data represent mean±SEM with n=7 to 8.

Fig. 4. Influence of longer exposure (4 h) to testosterone on the relaxation responses of porcine coronary artery rings to (A) bradykinin and (B) calcium ionophore A23187. Artery rings were treated for 4 h with vehicle ( ), or 4 h with 1 nM testosterone (○) before being contracted with 30 nM U46619 and cumulatively relaxed with either bradykinin or A23187. Data represent mean±SEM with n=5 to 7 in each case. *P<0.05 when compared to corresponding vehicle-treated rings (ANOVA with post-hoc Bonferroni’s test).
Fig. 5. Effect of anti-androgens on the inhibitory influence of testosterone (1 nM) on endothelium-dependent relaxation mediated by (A) bradykinin and (B) A23187. Porcine coronary artery rings were incubated with vehicle (○, ●), 10 μM flutamide (△) or 10 μM cyproterone acetate (▽) for 20 min before being incubated for 20 min with 1 nM testosterone (○, △, ▽) or vehicle (●). Following contraction with 30 nM U46619, cumulative relaxation curves to bradykinin and A23187 were constructed. Data represent mean±SEM with n=7 to 8. *P<0.05 when compared to vehicle-treated rings (ANOVA with post-hoc Bonferroni’s test).

Fig. 6. Effect of transcriptional/translational inhibitors on the inhibitory influence of testosterone (1 nM) on endothelium-dependent relaxation mediated by (A) bradykinin and (B) A23187. Porcine coronary artery rings were incubated with vehicle (○, ●), 10 μM actinomycin D (△) or 10 μM cycloheximide (▽) for 20 min before being incubated for 20 min with 1 nM testosterone (○, △, ▽) or vehicle (●). Following contraction with 30 nM U46619, cumulative relaxation curves to bradykinin and A23187 were constructed. Data represent mean±SEM with n=7 to 8. *P<0.05 when compared to vehicle-treated rings (ANOVA with post-hoc Bonferroni’s test).

Table 2
Log EC_{50} values of bradykinin and A23187 in U46619-contracted porcine coronary artery rings following 20 min incubation with ethanol vehicle or 1 nM testosterone±10 μM flutamide/10 μM cyproterone acetate

<table>
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<tr>
<th>Relaxing agent</th>
<th>n</th>
<th>Vehicle</th>
<th>−Log EC_{50} (M)</th>
<th>Testosterone</th>
<th>Testosterone + flutamide</th>
<th>Testosterone + cyproterone acetate</th>
</tr>
</thead>
<tbody>
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<td>8.08±0.04</td>
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<td>7.88±0.01*</td>
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<td></td>
<td></td>
<td>[8.3]</td>
<td>[18.7]</td>
<td>[13.1]</td>
</tr>
<tr>
<td>A23187</td>
<td>7</td>
<td>7.23±0.06</td>
<td></td>
<td>6.97±0.04*</td>
<td>7.04±0.04</td>
<td>7.2±0.05*</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[58.5]</td>
<td>[106.9]</td>
<td>[91.4]</td>
</tr>
</tbody>
</table>

* Data represent mean±SEM with n indicating the number of hearts investigated. Log EC_{50} values are calculated from concentration-response relationships illustrated in Fig. 5. Mean EC_{50} values are denoted in square brackets. Statistical analysis was performed using ANOVA followed by Bonferroni’s test. *P<0.05 compared with corresponding vehicle control value. **P<0.05 compared with corresponding value for rings treated with 1 nM testosterone.
Table 3

Log EC\textsubscript{50} values of bradykinin and A23187 in U46619-contracted porcine coronary artery rings following 20 min incubation with ethanol vehicle or 1 nM testosterone: 10 \mu M actinomycin D/10 \mu M cycloheximide\textsuperscript{a}

<table>
<thead>
<tr>
<th>Relaxing agent</th>
<th>n</th>
<th>Vehicle</th>
<th>Testosterone</th>
<th>Testosterone + flutamide</th>
<th>Testosterone + cyproterone acetate</th>
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<td>8.05±0.02</td>
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<td>7.75±0.05*</td>
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<td>[8.9]</td>
<td>[19.7]</td>
<td>[18.1]</td>
<td>[14.9]</td>
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<tr>
<td>A23187</td>
<td>7</td>
<td>7.24±0.01</td>
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<td>7.03±0.06*</td>
<td>6.92±0.05*</td>
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<tr>
<td></td>
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<td>[58.2]</td>
<td>[106.9]</td>
<td>[94.0]</td>
<td>[119.1]</td>
</tr>
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</table>

\textsuperscript{a}Data represent mean±SEM with n indicating the number of hearts investigated. Log EC\textsubscript{50} values are calculated from concentration-response relationships illustrated in Fig. 6. Mean EC\textsubscript{50} values are denoted in square brackets. Statistical analysis was performed using ANOVA followed by Bonferroni’s test. *P<0.05 compared with corresponding vehicle control value.

nM) of testosterone attenuated endothelium-dependent vasorelaxation by bradykinin and calcium ionophore A23187 while not affecting levromakalim- and sodium nitroprusside-induced endothelium-independent effects. Consistent with earlier work using supraphysiological hormone concentrations [20,21], we also observed that testosterone can itself cause relaxation independent of the endothelial layer when applied at concentrations in the higher, micromolar range. Hence, the most striking feature of the current work is that although supraphysiological concentrations of testosterone were required to elicit direct relaxation, lower, nanomolar concentrations of the male sex hormone were able to modulate endothelium-dependent relaxation. Furthermore, this effect was observed after both 20 min and 4 h exposures to 1 nM testosterone. Based on the results obtained in the presence of the testosterone receptor antagonists flutamide and cyproterone acetate, it would appear that the testosterone receptor does not participate in the direct relaxing effect. In contrast, this steroid receptor appears to be partially responsible for the indirect modulatory actions of testosterone. Our data also suggest that the direct and indirect effects of testosterone may be due to non-genomic actions as these responses were insensitive to actinomycin D and cycloheximide.

The disproportion in CAD incidence observed in men and women of all ages [2] has resulted in speculations that endogenous and/or exogenous estrogens confer some form of cardioprotection on women [5,6]. Direct evidence for this causal link is however limited. Nevertheless, the current consensus is that estrogen favorably regulates serum lipoprotein levels [22,23], acts as an antioxidant [24,25], and exerts direct vascular influences [26,27].

Interestingly, somewhat less attention has been paid to the alternative possibility that circulating levels of androgens could prime men for increase CAD incidents. In animals, prolonged treatment with physiological doses of testosterone alters hemodynamic and vascular properties. The norepinephrine-induced vasopressor effect in testosterone-treated, castrated male rats was raised significantly [28] as was the sensitivity of arteries from male pigs [29] and rodents [30,31] to contractile agents. In contrast, clinical evidence demonstrating that physiological or excessive amounts of androgens could be harmful to the human vasculature has only surfaced recently in a handful of study groups. Female-to-male transsexuals on long-term, high-dose androgen therapy exhibited worse flow-mediated vasodilatation than their normal control group [17]. Recently, it was reported that men who had undergone at least 6 months of complete androgen deprivation for prostate cancer treatment displayed significantly better arterial endothelial function than the control subjects who had normal levels of androgen [18]. Taken together these data, coupled with reports of cardiovascular incidents associated with androgenic-anabolic steroid abuse [7,8] support the deleterious role of testosterone on the vascular system.

In our in vitro preparation, we observed testosterone-induced, concentration-dependent relaxation in the porcine coronary artery. This effect was endothelium-independent and prostacyclin was not involved in this direct testosterone-induced response since the cyclooxygenase inhibitor indomethacin was always present in the bathing medium. The potential time-dependence of this interaction was addressed by experiments where ring segments were exposed to testosterone for a longer, 4 h incubation. Longer treatments were not attempted in order to preclude the involvement of gene-mediated events, while shorter exposures to testosterone were inappropriate in light of the time required for each experimental protocol (~2 h). Our data indicate that this phenomenon was not time-dependent as the observed effect after 20 min and 4 h exposures were similar. The anti-androgens, flutamide and cyproterone acetate, as well as the transcription/translation inhibitors, actinomycin D and cycloheximide, were unable to block this direct relaxing effect. This was not surprising since the rapid time frame within which the response was observed is uncharacteristic of actions occurring via gene-mediated events.

In accord with our observations, Costarella et al. [20] and Yue et al. [21] also demonstrated that high concentrations (\mu M) of testosterone can evoke concentration-dependent, endothelium-independent relaxation in agonist-contracted artery rings. These authors further suggest a beneficial role for testosterone in vascular tissues and
extrapolate their findings to clinical reports indicating the therapeutic potential of testosterone for treating cardiovascular disorders. These in vitro observations, however, were dependent on unrealistic concentrations of testosterone far removed from those found in the circulation. In addition to testosterone, various steroids have been shown to cause direct vasodilatation including 17β-estradiol [27], 17α-estradiol [32] and progesterone [33]. The lack of specificity and the supraphysiological (micromolar) steroid concentrations required, however, brings to question the clinical relevance of these functional investigations. Indeed, since the concentrations of testosterone required for such direct relaxation responses were over a 100-fold greater than those detected under physiological conditions in men (10–38 nM) [34], it would therefore appear that these direct effects would only be of pharmacological relevance. More importantly, these studies do not address the issue of how hormones may influence vaso-motor function in a more physiological setting. Our findings that vasorelaxation to bradykinin and A23187 are impaired in the presence of low concentrations of testosterone are consistent with the concept that this steroid exerts a deleterious influence on vascular function, probably through a specific receptor-mediated response.

There has been several indications of androgen receptors in blood vessels [35,36] implicating the cardiovascular system as a candidate for androgen activities. The modulatory events of testosterone were apparent at concentrations comparable to those in the circulation and as such the phenomenon we report here is concordant with receptor-mediated events. However, these effects were detected following only acute exposure to the male sex steroid and hence contrast with the typical slow-acting occurrences associated with nuclear steroid hormone receptors. Our results demonstrating the incomplete effectiveness of the androgen receptor antagonists, flutamide and cyproterone acetate, at reversing this outcome suggest that the classical steroid receptor may not be the only participating receptor in this response. Indeed the existence of fast-acting membrane steroid receptors has been reported in neuronal cells [37].

The porcine hearts used in the current work were from a local abattoir and we were unable to determine or control the sex distribution. While we recognize this as a limitation of our study, the data presented were reproducible in every batch of hearts studied. As such, it is unlikely that the response to testosterone we observed in our model is dependent on the gender of the pigs.

In conclusion, we have found that while supraphysiological levels of testosterone produce endothelium-independent relaxation in porcine coronary artery rings, lower amounts of the male sex steroid can modulate endothelium-dependent relaxation. The direct and indirect effects of testosterone on vasorelaxation were, respectively, insensitive and partially reversed by the androgen receptor antagonists. However, the possibility that they were mediated via different non-classical steroid receptors remains to be determined. Since the model used in this study is not amenable to genetic manipulations, further mechanistic explorations will likely require other techniques. As impaired endothelium-dependent vasodilatation is associated with CAD, our data may account for some of the suggested detrimental activities of testosterone on the vasculature.

Acknowledgements

We thank Godfrey S.K. Man for excellent technical assistance. This study was supported in part by a Committee on Research and Conference Grant and a post-doctoral fellowship (HT) from the University of Hong Kong. All authors are members of the Institute of Cardiovascular Science and Medicine, University of Hong Kong.

References