Attenuated $\beta$-adrenoceptor-mediated cardiac contractile responses following androgenic steroid administration to sedentary rats

Abstract Androgenic steroids administered in doses at pharmacological levels to sedentary animals have been shown to result in a reduced $\beta$-adrenoceptor-mediated increase in systolic cardiac performance when assessed in vivo. Whether the attenuated adrenergic response occurs as a consequence of alterations in either cardiac loads, heart rate, modifications in left ventricular (LV) geometry, or a decrease in myocardial contractile performance has not been determined. In this study the effect of chronic administration (over 3 months) of an androgenic steroid (nandrolone decanoate, 5 mg $\cdot$ kg$^{-1}$ biweekly) on the response of load-insensitive indices of myocardial contractile function [the slope of the LV systolic stress-strain relationship ($LV-E_{\text{max}}^{\text{n}}$, where $E_{\text{max}}^{\text{n}}$ is systolic myocardial elastance)] to an adrenergic-inotropic stimulus was examined ex vivo in paced rat hearts. Systolic cardiac performance was assessed at 300 beats $\cdot$ min$^{-1}$ in isolated constant flow perfused heart preparations both before and during $10^{-8.5}$ mol $\cdot$ l$^{-1}$ isoproterenol (ISO) infusion (approximate concentration of ISO eliciting 50% maximal inotropic response to ISO). Steroid administration resulted in left-shifted LV systolic and diastolic pressure-volume ($P$-$V$) relationships. The left-shifted $P$-$V$ relationships were attributed, in part, to increased slopes of these relationships. However, the steroid-mediated increment in the slope of the systolic $P$-$V$ relationship (systolic chamber elastance, $E_{\text{max}}$) was not associated with a similar change in LV $E_{\text{max}}^{\text{n}}$ [control 19.2 (SEM 2.1) g $\cdot$ cm$^{-2}$, steroid 18.3 (SEM 2.4) g $\cdot$ cm$^{-2}$] as determined in the absence of ISO. Isoproterenol infusion resulted in an increase in both $E_{\text{max}}$ and $E_{\text{max}}^{\text{n}}$ in the control rats, without altering systolic performance in the steroid treated rats. Consequently, in the presence of ISO, the steroid treated rats exhibited a similar $E_{\text{max}}$, but a reduction in $E_{\text{max}}^{\text{n}}$ compared to the control rats [control 25.6 (SEM 1.9) g $\cdot$ cm$^{-2}$, steroid 18.5 (SEM 1.5) g $\cdot$ cm$^{-2}$; $P < 0.05$]. In conclusion, these results would suggest that chronic high dose androgenic steroid administration produces a decrease in myocardial contractile reserve to $\beta$-adrenoceptor stimulation.

Key words Steroids $\cdot$ Inotropy $\cdot$ $\beta$-Adrenoceptor agonist

Introduction

It has been reported that androgenic-anabolic steroids are synthesised for use in modern medicine for therapeutic reasons (Hoberman and Yesalis 1995). However, athletes have been reported as abusing androgenic-anabolic steroids at supraphysiological doses to improve physical performance through a purported effect on muscle mass and strength (Crist et al. 1983; Brower et al. 1990; Titlestad et al. 1994). Over the past decade attention has been focussed on the potential cardiovascular side effects of abuse of androgenic steroids, following case reports of cardiovascular mortality and morbidity in young athletes using androgenic steroids (McNutt et al. 1988; Luke et al. 1990). Potential effects of the abuse of androgenic steroids on cardiac performance have been suggested. Although, it has been shown that systolic left ventricular (LV) performance is normal in weight lifters using androgenic steroids (Pearson et al. 1986; Urhausen et al. 1989), the current non-invasive techniques for determining cardiac performance in humans do not allow for an assessment of load and heart-rate-insensitive measures of performance during similar degrees of adrenergic activation. In addition, in human studies a confounding variable is being unable to assess accurately the degree and duration of
the abuse of androgenic steroids and to take into account the influence of exercise training programmes on cardiac geometry and performance.

Despite the limitations of assessing the effect of androgenic steroids on cardiac performance in human studies, there have been few reports examining the influence of supraphysiological doses of androgenic steroids on cardiac function in either sedentary or exercise-trained animals. We have recently described an influence of androgenic steroids on cardiac diastolic performance in sedentary rats (Trifunovic et al. 1995, 1998). However, data on systolic performance obtained in vivo in rats by our group, although tending to show a reduced performance in steroid-treated sedentary animals was limited by our inability to construct systolic pressure-dimension relationships over a low range of filling volumes (Trifunovic et al. 1995). Over a low range of filling volumes we (Trifunovic et al. 1995) would have risked having to account for an influence of reduced coronary flows on systolic cardiac performance. Nevertheless, Liang et al. (1993) have shown that administration of an androgenic steroid in high doses decreases a load-insensitive measure of cardiac systolic performance in exercise-trained, but not sedentary rats. Alternatively, Ramo (1987) has shown a reduced β-adrenoceptor-mediated change in systolic cardiac performance in sedentary dogs receiving high doses of an androgenic steroid. A detrimental action of androgenic steroids in high doses on adrenergic-mediated increases in systolic cardiac performance described by Ramo (1987) could be ascribed to alterations in the conditions of cardiac loading or heart rate. At present there are no data examining the effect of administering androgenic steroids in high doses on adrenergic-inotropic responses in sedentary animals using load-insensitive measures of systolic performance at controlled heart rates.

The aim of our study was therefore to determine the effect of chronic administration of a commonly used androgenic steroid, nandrolone decanoate, on LV contractile performance in the presence of an adrenergic-stimulus in sedentary animals.

### Methods

**Experiment groups**

The current study was approved by the University of the Witwatersrand Animal Ethics Committee (number 96/08/5). A group of 19, 250-g male Sprague Dawley rats was assigned to two groups, a steroid-treated group (steroid-treated group = 10; control group = 9). The steroid-treated group received an injection of nandrolone decanoate (ester-4-en-3-one, 17β-hydroxy-17α-acetoxy-4-ene-3-decanoate, Deca Durabolin, Organon), as previously described (Trifunovic et al. 1995, 1998). This dose is comparable to the dose that has been reported as being frequently used by athletes – 600 mg · week⁻¹ or approximately 8 mg · kg⁻¹ · week⁻¹ (Pope and Katz 1988). The control group received a weekly injection of arachis oil with 10% (vol/vol) benzyl alcohol, the vehicle for the androgenic steroid. Steroid and vehicle administration continued for a 3-month period during which time the rats received standard laboratory rat chow and water ad libitum. All the rats were housed in a room that was lighted between 0600 and 1800 hours.

**Isolated perfused heart preparation**

At the end of the 3-month steroid and vehicle treatment period, the rats were anaesthetised with ketamine (75 mg · kg⁻¹) and xylazine (15 mg · kg⁻¹) and their hearts were excised and immediately rinsed in an ice-cold physiological saline solution (PSS). Hearts were perfused retrogradely at a constant flow with 37°C PSS containing: NaCl 118, KCl 4.7, NaHCO₃ 25, KH₂PO₄ 1.2, MgSO₄ 1.2, and glucose 10 mmol · l⁻¹ at a pH of 7.40 gassed with a 95% O₂:5% CO₂ mixture. The coronary flow rate was determined volumetrically and adjusted to achieve an approximate volume flow of 12 ml · min⁻¹ · g⁻¹ heart mass, according to the estimated mass of the heart as measured immediately after excision, with large vessels and pericardium still attached. The coronary perfusion pressure was monitored from a side-arm of the aortic perfusion cannula with a Statham P23 transducer. The hearts were paced at 300 beats · min⁻¹ with the voltage 10% above threshold, via platinum wire electrodes attached to the left atrium and the apex of the heart.

Peak (maximal) systolic LV pressure (LVPmax) and LV diastolic pressure (LVDP) were determined using a water-filled balloon-tipped cannula coupled to a Gould P50 pressure transducer, inserted via the left atrium into the LV cavity. A thin-walled latex balloon with a filling volume beyond maximal LV lumen capacities was selected for this study to avoid the stiffness of the balloon wall contributing toward LVP at higher filling volumes. The volume of the balloon wall was assessed using a water displacement technique and the same balloon was used throughout the study. The LV pressure and coronary perfusion pressures were recorded using high-impedance polygraph print-out. An incremental increase in LV volumes gradually to values which resulted in LVDP of between 20–30 mmHg. The LV pressures were determined at as many multiple small increments in volume as were practically possible to improve on the accuracy of curve fitting during later analysis.

Following the determination of LVPmax-volume relationships in the absence of an isotropic stimulus, the LV balloon was emptied. Isoproterenol (ISO, Sigma, 10⁻⁶ mol · l⁻¹) was infused at 1% of the coronary flow rate into the PSS perfusate to provide a final concentration of 10⁻⁸ mol · l⁻¹. During a constant ISO infusion, approximately 10 min from the start of the infusion when the increase in LV developed pressures had stabilised, LV balloon volumes were again increased and LV systolic pressures recorded using the same approach as described above. In a pilot study conducted in control rats, we had found that higher concentrations of ISO resulted in marked increases in systolic pressures at the lowest balloon volumes obtainable. The consequence had been an inability to construct meaningful systolic performance relationships. Therefore, for the current study we chose a concentration of ISO determined to be the approximate EC₅₀ (concentration of ISO that elicited 50% of the maximal inotropic response). An isotropic response (LV +dP/dt response) to 20-s infusions of eight incremental ISO concentrations ranging from 6.6 × 10⁻¹⁰ mol · l⁻¹ to 10⁻⁷ mol · l⁻¹ in hearts from 21 control rats. We have previously determined that 20-s infusions of ISO are sufficient to produce maximal increases in LV +dP/dt (Norton et al. 1999).

The LV systolic chamber performance both before and during ISO treatment was determined from the slopes of the LV systolic volume (LV max-volume, V) relationship (systolic chamber elastance, Eₛ₃ₒ₃; see Webster et al. 1988). To account for differences in LV geometry, LV systolic performance was also assessed from the slope of the line-approximated systolic σ-strain relationship (myocardial systolic function or systolic myocardial elastance, Eₛ₃ₒ₃; see Webster et al. 1988). Systolic σ and strain were calculated from previously described equations (Webster et al. 1988), assuming a thick-walled, spherical LV geometry, as follows: σ (grams per centimetre squared) = 1.36 ×