

Cardiac concentric remodelling induced by non-aromatizable (dihydro-)testosterone is antagonized by oestradiol in ovariectomized rats

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Abstract

Previous studies on the cardiovascular effects of androgens in females, most of them using testosterone treatment, have yielded conflicting results. Testosterone is metabolized into oestradiol (E_2) and dihydrotestosterone (DHT) within cardiovascular tissues. The aim of the present study was to explore the cardiovascular effects exerted by E_2 and the non-aromatizable androgen DHT and to study possible interactions between these in female rats. Ovariectomized rats were treated with DHT, E_2 , or DHT+ E_2 for 6 weeks. DHT increased left-ventricular posterior wall thickness, assessed by echocardiography, whereas left-ventricular dimension, as well as total heart weight and calculated left-ventricular mass, were unchanged. DHT also increased the levels of insulin-like growth factor-I mRNA in the left ventricle. E_2 abolished the effect of

DHT on left-ventricular remodelling and insulin-like growth factor-I mRNA when the two treatments were given in combination. E_2 also reduced androgen receptor mRNA levels in the heart. Neither E_2 nor DHT changed blood pressure measured by telemetry. In conclusion, treatment with the endogenous non-aromatizable androgen DHT causes cardiac concentric remodelling in ovariectomized rats, possibly mediated by increased local levels of insulin-like growth factor-I. The effect of DHT on cardiac wall thickness was antagonized by E_2 , possibly through downregulation of cardiac androgen receptors. These mechanisms may be of importance for the concentric left-ventricular geometric pattern developing in women after menopause.

Journal of Endocrinology (2006) **189**, 485–491

Introduction

Sex steroids are of profound physiological and pathophysiological importance within the female cardiovascular system. There are numerous studies reporting beneficial effects of oestrogens on serum lipids, vascular function and experimental atherosclerosis in females (Hodgin & Maeda 2002, Mendelsohn & Karas 2005). Oestrogens have also been suggested to modulate cardiac mass and attenuate the development of cardiac hypertrophy in females (van Eickels *et al.* 2001, Hayward *et al.* 2001, Babiker *et al.* 2004, Pelzer *et al.* 2005). In contrast to these results, recent clinical trials have failed to detect any cardiovascular benefit, or have even shown adverse effects, of hormone-replacement therapy in postmenopausal women (Nelson *et al.* 2002, Barrett-Connor 2003).

While many studies have focused on the effects of oestrogens in females, the effects of androgens have been less studied, and even less so the possible interactions between oestrogens and androgens. In females, androgens are secreted by both the ovaries and the adrenal glands and

have been suggested to be of importance for libido, bone mineral density, and muscle mass and strength (Snyder 2001). Previous studies of the effects of androgens on the female cardiovascular system, most of them using testosterone treatment, have suggested beneficial as well as detrimental effects on vasoreactivity and atherogenesis (Liu *et al.* 2003, Wu & von Eckardstein 2003). These conflicting results may be partly explained by the complexity of sex steroid biology, including local and systemic metabolism of sex steroids. Testosterone is readily aromatized into oestradiol (E_2), and 5α -reduced into the more potent and non-aromatizable androgen 5α -dihydrotestosterone (DHT) within cardiovascular tissues (Thum & Borlak 2002, Liu *et al.* 2003). Therefore, the observed net effect of testosterone administration is highly dependent upon enzyme activity of aromatase and 5α -reductase and includes both an oestrogenic and an androgenic component.

The aim of the present study was to explore the cardiovascular effects exerted by E_2 and the endogenous non-aromatizable androgen DHT, respectively, and to

study possible interactions between these in female rats. We have treated ovariectomized (OVX) rats with E₂, DHT or the combination for 6 weeks and studied the effects on cardiac structure and performance (assessed by echocardiography) and blood pressure (telemetry).

Materials and Methods

Animals and study protocol

Female Sprague–Dawley rats (Scanbur BK AB, Sollentuna, Sweden) were housed in a temperature- and humidity-controlled room with a 06:00–18:00 h light regime and allowed a soy-free diet (R70; Lactamin AB, Kimstad, Sweden) and tap water *ad libitum*. All procedures were approved by the ethics committee at Göteborg University, Sweden and conformed to the UFAW Handbook on the Care and Management of Laboratory Animals.

The animals were randomly divided into five groups: sham operation+vehicle treatment (Sham; $n=12$), OVX+vehicle treatment (V; $n=10$), OVX+17 β -oestradiol treatment (E₂; $n=11$), OVX+DHT treatment (DHT; $n=11$) and OVX+combined E₂ and DHT treatment (E₂+DHT; $n=11$). At 12 weeks of age (body weight 251 ± 2 g) the rats were either sham-operated or OVX under isoflurane anaesthesia (Baxter Medical AB, Kista, Sweden) and small silastic implants were placed subcutaneously in the cervical region. The silastic implants were prepared as previously described (Vandenput *et al.* 2002), releasing 2.5 μ g/day E₂ or 40 μ g/day DHT (Sigma Chemical Co). Vehicle-treated animals received an empty implant and the E₂+DHT group received both an E₂ and a DHT implant. After 4 weeks of treatment, radio-telemetry transmitters were implanted ($n=8$ /group, see below). After 6 weeks of treatment, the rats were examined by echocardiography ($n=8$ /group, see below) and killed by excision of the heart; the uterus and tibia were then collected. Tibia length was determined as described by Tivesten *et al.* (2004) and heart weight was normalized for body weight as well as tibia length (Yin *et al.* 1982). The results on bone variables in these animals have been published previously (Tivesten *et al.* 2004).

Serum hormones

Commercially available RIA kits (Diagnostic Systems Laboratories, Webster, TX, USA) were used to assess serum concentrations of testosterone (DSL-4100), E₂ (third-generation E₂ DSL-39100) and DHT (DSL-9600) at the end of the study.

Telemetric blood pressure and heart-rate measurements

The rats were anaesthetized using sodium pentobarbital (60 mg/kg; Apoteksbolaget, Umeå, Sweden) and

a telemetry transmitter catheter (outer diameter 0.76 mm; Data Sciences International, St Paul, MN, USA) was implanted into the abdominal aorta and glued into position (Super-Attak; Loctite, Göteborg, Sweden). The transmitter (TA11PA-C40) was secured to the abdominal wall and the abdomen closed with sutures. After 1 week of recovery, the signal was collected using the Dataquest LabPRO Acquisition System (version 3.1; Data Sciences International). The blood-pressure signal was corrected for electronic offset; that is, the average of a measurement outside the animal before implantation and after explantation. Systolic, mean arterial and diastolic blood pressure and heart rate were recorded over two consecutive nights (20:00–06:00 h).

Echocardiography

Cardiac ultrasound studies were performed as previously described (Tivesten *et al.* 2000) using a commercially available ultrasonograph (ATL, HDI 5000 SonoCT; Philips Ultrasound, Bothell, Seattle, WA, USA) equipped with a 15 MHz linear array transducer. The rats were anaesthetized (isoflurane; Baxter Medical AB) and the anterior chest was shaved. A warming pad was used to maintain body temperature. A long-axis view of the left ventricle was obtained and, perpendicular to this, a two-dimensional short-axis view of the left ventricle at the level between the papillary muscles and mitral valve. This served as a guide for M-mode tracings. By superior angulation in the short-axis view the pulmonary artery was visualized and pulsed-wave Doppler signals (5 MHz) were obtained. Pulsed-wave Doppler spectra of mitral inflow were recorded from an apical four-chamber view.

Images were stored in the DICOM format and off-line measurements were performed by two independent observers (Å T, E B) using an image-analysis system (EchoPAC 6.3; GE Medical Systems, West Milwaukee, WI, USA). M-mode measurements of left-ventricular internal diameters and posterior-wall thickness in diastole and systole were made using the leading-edge convention recommended by the American Society of Echocardiography (Sahn *et al.* 1978). At least three beats were averaged for each measurement. Fractional shortening was calculated as follows: (left-ventricular internal diameter in diastole – systolic left-ventricular internal diameter)/diastolic left-ventricular internal diameter \times 100%. Relative wall thickness was calculated as (2 \times diastolic posterior wall thickness)/diastolic left-ventricular internal diameter. Left-ventricular mass was calculated as described by Pawlusch *et al.* (1993).

Doppler measurements included pulmonary artery Doppler flow and peak early (E) and late (A) mitral inflow velocities. Measurements of pulmonary artery diameter were performed at the level of the sampling site from digitized cine loops in three consecutive heartbeats. Stroke volume was calculated as the product of the

Table 1 Serum hormones and body and organ weights

	Sham	V	E ₂	DHT	E ₂ +DHT	P (ANOVA)
Serum testosterone (ng/ml)	0.298 ± 0.056	0.017 ± 0.005 ^a	0.032 ± 0.005 ^a	0.024 ± 0.004 ^a	0.041 ± 0.005 ^a	<0.0001
Serum DHT (ng/ml)	0.074 ± 0.005	0.059 ± 0.004	0.035 ± 0.003	0.195 ± 0.020 ^{abc}	0.198 ± 0.029 ^{abc}	<0.0001
Serum E ₂ (pg/ml)	23.0 ± 5.1	10.8 ± 1.5	39.6 ± 8.1 ^{ab}	7.3 ± 1.5 ^{ac}	48.3 ± 6.6 ^{abd}	<0.0001
Body weight (g)	286 ± 6	330 ± 9 ^a	268 ± 4 ^{ab}	329 ± 10 ^{ac}	277 ± 4 ^{bd}	<0.0001
Uterine weight (mg)	529 ± 48	145 ± 10 ^a	923 ± 116 ^{ab}	186 ± 16 ^{ac}	867 ± 37 ^{abd}	<0.0001
Heart weight (mg)	929 ± 18	1011 ± 30 ^a	887 ± 17 ^b	1004 ± 24 ^{ac}	839 ± 17 ^{abd}	<0.0001
Heart weight/BW (mg/100 g)	325 ± 6	306 ± 6 ^a	332 ± 6 ^b	304 ± 7 ^{ac}	303 ± 5 ^{ac}	0.001
Heart weight/TL (mg/mm)	23.7 ± 0.4	25.1 ± 0.6 ^a	23.3 ± 0.4 ^b	25.0 ± 0.6 ^{ac}	21.6 ± 0.5 ^{abcd}	<0.0001

Twelve-week-old female rats were OVX and then treated with vehicle (V), E₂, DHT or the combination (E₂+DHT) for 6 weeks. Sham-operated animals were also included (Sham); n=10–12. Values are given as means ± S.E.M. ^aP<0.05 versus sham; ^bP<0.05 versus vehicle; ^cP<0.05 versus E₂; ^dP<0.05 versus DHT (ANOVA followed by Fisher's protected least significant difference test). BW, body weight; TL, tibia length.

velocity time integral (mean of three consecutive pulmonary artery velocity profiles) and the corresponding pulmonary artery area. Multiplying stroke volume by heart rate yielded cardiac output. Left-ventricular diastolic function was estimated by the mitral filling (E/A) ratio.

Real-time PCR

RNA was extracted from the left ventricle using Trizol reagent (Invitrogen), purified using the RNeasy kit (Qiagen) and reverse-transcribed into cDNA. The PCR analysis was performed using the ABI Prism 7000 Sequence Detection System (Applied Biosystems, Stockholm, Sweden) with specific probes labelled with the reporter fluorescent dye FAM. The sequences for forward and reverse primers and probe for rat insulin-like growth factor-I (IGF-I) mRNA (accession number X06043) were 5'-CAGGCTATGGCTCCAGCATT-3' (nucleotides 95–114), 5'-GCTCCGGAAGCAACACTCAT-3' (nucleotides 162–143) and 5'-AGGGCACCACAGACGGGCATTGT-3' (nucleotides 118–140), respectively. For the androgen receptor (AR) and oestrogen receptor- α (ER α) mRNA quantification, pre-designed primers and probes were used (TaqMan[®] Gene Expression Assay, IDs Rn00560747_m1 and Rn00562166_m1, respectively). The cDNA-amplification conditions were 50 °C for 2 min and 95 °C for 10 min, followed by 40 cycles at 95 °C for 15 s and 60 °C for 1 min, and the mRNA amount of each gene was adjusted for the levels of β -actin mRNA (TaqMan[®] Gene Expression Assay, ID Rn00667869_m1). The oligonucleotide primers and probes were all supplied by Applied Biosystems.

Statistical analysis

Data are presented as means ± S.E.M. A P value of <0.05 was considered statistically significant. Data were initially analyzed using one-way ANOVA and, when P<0.05, by *post hoc* comparisons using Fisher's protected least-significant difference test. AR and ER α mRNA data from

the four OVX groups were analyzed using a two-way ANOVA with respect to the effects of E₂ and DHT treatment. Data collected with telemetry were analyzed using an ANOVA adapted for repeated measures. All statistical analyses were performed using Statview software (Abacus Concepts, Berkeley, CA, USA).

Results

Serum hormones and body and organ weights

In OVX rats treated with vehicle, serum concentrations of testosterone were only 6% of the levels in the sham-operated rats, and these were not changed by DHT or E₂ treatment (Table 1). Serum DHT levels were not changed by ovariectomy, while DHT treatment increased serum DHT to levels approximately 2.6-fold higher than those of sham rats. The reduction of serum E₂ levels by ovariectomy did not reach statistical significance. In the two groups on E₂ treatment, serum E₂ levels were higher than the levels of sham rats. Serum concentration of DHT was not changed by the addition of E₂, and serum concentration of E₂ was not significantly changed by the addition of DHT in the group with combined E₂+DHT treatment.

As expected, ovariectomy resulted in increased body weight. This was prevented by E₂ treatment, while DHT treatment did not change body weight as compared with vehicle treatment (Table 1). The E₂ dose chosen prevented the ovariectomy-induced loss in uterine weight and even resulted in higher uterine weight as compared with sham rats, while DHT did not affect the uterine weight.

Total heart weight was increased by ovariectomy, and E₂ treatment fully prevented this effect (Table 1). Heart weight adjusted to body weight was reduced in OVX compared with sham rats and increased by E₂ compared with vehicle. In comparison, heart weight adjusted to tibia length was increased in OVX compared with sham rats, reduced by E₂ and further reduced in the E₂+DHT group. DHT alone had no effect on either unadjusted or

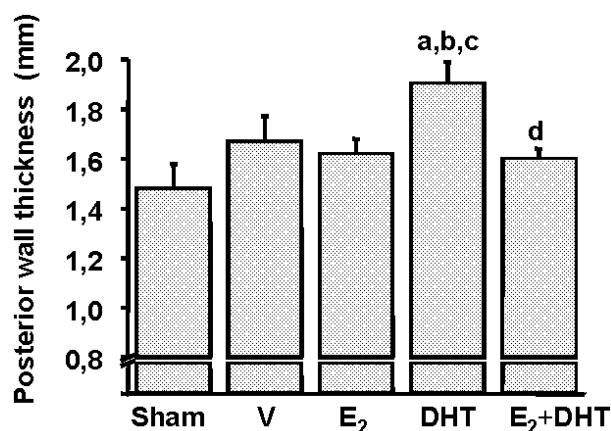


Figure 1 Posterior wall thickness of the left ventricle in diastole. Twelve-week-old female rats were OVX and then treated with vehicle (V), E₂, DHT or the combination (E₂+DHT) for 6 weeks. Sham-operated animals were also included (Sham); $n=7-8$. Values are given as means \pm S.E.M. $P(\text{ANOVA})=0.013$. ^a $P<0.05$ versus sham; ^b $P<0.05$ versus vehicle; ^c $P<0.05$ versus E₂; ^d $P<0.05$ versus DHT (post hoc analysis by Fisher's protected least significant difference test).

body weight- or tibia length-adjusted heart weight as compared with vehicle.

Cardiac structure and function assessed by echocardiography

To investigate the effects of the different treatments on cardiac structure and function, echocardiography was performed after 6 weeks of treatment. Compared with vehicle treatment, DHT increased the posterior wall thickness of the left ventricle (Fig. 1). No effect was seen for E₂ treatment alone; however, the addition of E₂ to DHT counteracted the stimulatory effect of DHT on left-ventricular wall thickness. Moreover, E₂ treatment reduced the calculated left-ventricular mass in DHT-treated rats (Table 2). There was no statistically significant

effect of either treatment on left-ventricular dimensions in systole or diastole, relative wall thickness or fractional shortening (Table 2). Cardiac output adjusted for body weight was increased by E₂, but unchanged by DHT treatment. There were no significant effects on heart rate or left-ventricular diastolic function (mitral E/A ratio).

Blood pressure and heart rate measured by telemetry

There were no differences between the groups in systolic, diastolic or mean arterial blood pressure or heart rate collected with telemetry (Table 3).

Cardiac IGF-I, AR and ER α mRNA levels

In an attempt to understand the mechanisms by which cardiac wall thickness was affected, real-time quantitative PCR analyses of IGF-I, AR and ER α mRNA levels in the left ventricle were performed. In line with the effect on posterior left-ventricular wall thickness, levels of IGF-I mRNA were increased by DHT treatment, and the addition of E₂ to DHT counteracted the stimulatory effect of DHT on IGF-I mRNA levels (Fig. 2).

Based on the results that E₂ abolished the effect of DHT on cardiac wall thickness and IGF-I mRNA, we hypothesized that E₂ may reduce the expression of the AR in the heart. Indeed, E₂ reduced the mRNA levels of AR in the left ventricle (Fig. 3). E₂ treatment also downregulated ER α mRNA levels in the heart (Sham 0.69 ± 0.16 , vehicle 0.83 ± 0.10 , E₂ 0.33 ± 0.05 , DHT 0.82 ± 0.16 , E₂+DHT 0.50 ± 0.16 arbitrary units; effect of E₂ treatment in two-way ANOVA $P=0.004$). DHT affected the mRNA levels of neither the AR nor the ER α .

Discussion

In this study we have explored the cardiovascular effects of the endogenous non-aromatizable androgen DHT, with

Table 2 M-mode and Doppler echocardiography

	Sham	V	E ₂	DHT	E ₂ +DHT	P (ANOVA)
LV diameter _d (mm)	7.1 \pm 0.1	6.7 \pm 0.2	6.6 \pm 0.1	6.8 \pm 0.2	6.4 \pm 0.2	0.091
LV diameter _s (mm)	4.3 \pm 0.3	3.7 \pm 0.3	3.7 \pm 0.2	3.9 \pm 0.2	3.5 \pm 0.2	0.20
Relative wall thickness _d	0.42 \pm 0.03	0.50 \pm 0.04	0.49 \pm 0.02	0.56 \pm 0.04 ^a	0.50 \pm 0.02	0.043
LV mass (mg)	691 \pm 44	761 \pm 42	688 \pm 33	816 \pm 34 ^{ac}	613 \pm 30 ^{bd}	0.005
LV mass/BW (mg/100 g)	242 \pm 11	230 \pm 8	258 \pm 11	255 \pm 8	224 \pm 9	0.056
Fractional shortening (%)	39 \pm 4	44 \pm 4	44 \pm 2	42 \pm 2	45 \pm 2	>0.20
Heart rate (beats/min)	362 \pm 9	358 \pm 13	347 \pm 12	365 \pm 9	331 \pm 6	0.15
Cardiac output (ml/min)	125 \pm 8	113 \pm 9	130 \pm 7	117 \pm 8	127 \pm 7	>0.20
Cardiac output/BW (ml/min per kg)	438 \pm 32	351 \pm 28 ^a	489 \pm 37 ^b	365 \pm 22 ^c	466 \pm 26 ^{bd}	0.008
Mitral E/A ratio	1.49 \pm 0.10	1.49 \pm 0.14	1.45 \pm 0.13	1.32 \pm 0.10	1.68 \pm 0.13	>0.20

Twelve-week-old female rats were OVX and then treated as described in Table 1; $n=7-8$. Values are given as means \pm S.E.M. ^a $P<0.05$ versus sham; ^b $P<0.05$ versus vehicle; ^c $P<0.05$ versus E₂; ^d $P<0.05$ versus DHT (ANOVA followed by Fisher's protected least significant difference test). d, diastole; s, systole; LV, left-ventricular; BW, body weight; E/A, peak early (E) and late (A) mitral inflow velocities.

Table 3 Data from telemetry

	Sham	V	E ₂	DHT	E ₂ +DHT	P (ANOVA)
Systolic blood pressure (mmHg)	126 ± 3	126 ± 3	127 ± 3	123 ± 3	116 ± 6	>0.20
Diastolic blood pressure (mmHg)	85 ± 2	84 ± 2	77 ± 3	83 ± 3	81 ± 3	>0.20
Mean arterial blood pressure (mmHg)	103 ± 2	102 ± 2	100 ± 3	100 ± 3	97 ± 3	>0.20
Heart rate (beats/min)	378 ± 5	377 ± 5	378 ± 3	366 ± 6	367 ± 7	>0.20

Twelve-week-old female rats were OVX and then treated as described in Table 1; $n=7-8$. Values are given as means ± s.e.m.

and without concomitant E₂ treatment, in female OVX rats. We demonstrate that DHT treatment causes left-ventricular concentric remodelling in association with increased levels of IGF-I mRNA in the left ventricle. These effects were antagonized by E₂.

Concentric remodelling of the left ventricle may be defined, in relation to left-ventricular dimension, as an increased wall thickness without an increase in left-ventricular mass (Verdecchia *et al.* 1995). Left-ventricular concentric remodelling has been suggested to represent a distinct component of an early stage of development of cardiac hypertrophy and has been shown to be an important and independent predictor of cardiovascular risk in hypertensive patients (Verdecchia *et al.* 1995). In the present study, DHT increased posterior wall thickness, whereas left-ventricular dimension, as well as total heart weight and calculated left-ventricular mass, were unchanged. These alterations can be summarized as concentric remodelling; however, as blood pressure was unchanged by DHT treatment, the concentric remodelling in the present study is most likely induced by a direct trophic effect of DHT on the myocardium.

DHT has previously been shown to induce a hypertrophic response in cardiomyocytes from male rats *in vitro*

(Marsh *et al.* 1998) and administration of DHT has been suggested to induce cardiac hypertrophy in female rats (Tsunoda *et al.* 1999). Our finding that DHT increased the levels of IGF-I mRNA in the left ventricle is also in line with previous studies showing that testosterone may increase IGF-I mRNA in cardiac muscle in association with hypertrophy (Nahrendorf *et al.* 2003). IGF-I is known to induce a dose-dependent cardiac hypertrophy, may act as a mediator of cardiac remodelling/hypertrophy induced by pressure or volume overload and has been shown to be an independent determinant of left-ventricular mass and geometry in human hypertension (Isgaard *et al.* 1999, Verdecchia *et al.* 1999). Therefore, it is reasonable to suggest that local IGF-I may play a role as a mediator of the androgen-induced concentric remodelling found in the present study.

In the present study, E₂ alone had no effect on left-ventricular wall thickness and IGF-I, but abolished the effect of DHT on left-ventricular wall thickness and IGF-I mRNA when the two treatments were given in combination. As E₂ antagonized the effects of DHT, we hypothesized that E₂ may reduce the expression of cardiac AR expression. Indeed, we found that E₂ reduced the AR mRNA levels in the heart. Previously, both E₂ and phytoestrogens have been shown to reduce AR mRNA levels

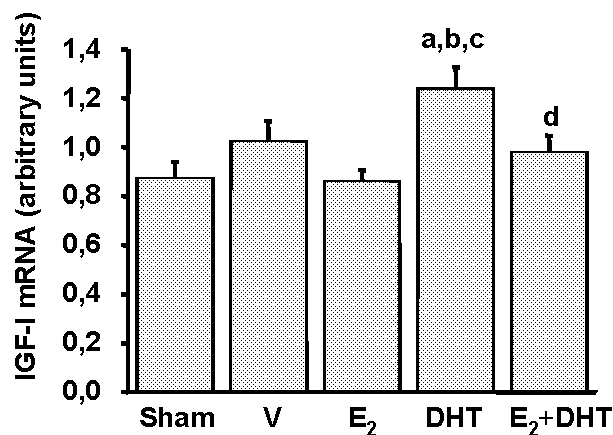


Figure 2 IGF-I mRNA levels in the left ventricle. Twelve-week-old female rats were OVX and then treated as described for Fig. 1; $n=10$. Values are given as means ± s.e.m. $P(\text{ANOVA})=0.006$. ^a $P<0.05$ versus sham; ^b $P<0.05$ versus vehicle; ^c $P<0.05$ versus E₂; ^d $P<0.05$ versus DHT (*post hoc* analysis by Fisher's protected least significant difference test).

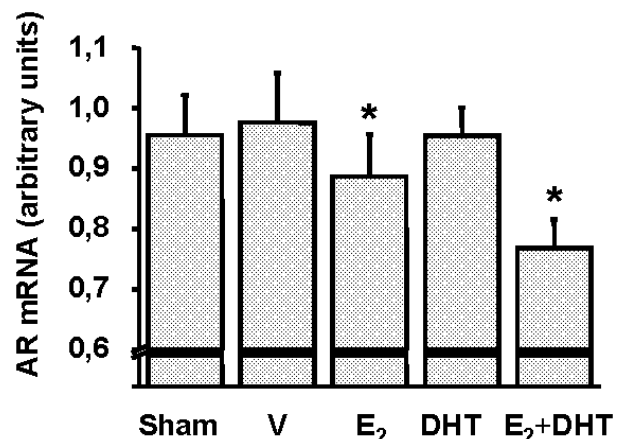


Figure 3 AR mRNA levels in the left ventricle. Twelve-week-old female rats were OVX and then treated as described for Fig. 1; $n=10-12$. Values are given as means ± s.e.m. *Effect of E₂ treatment ($P<0.05$) in two-way ANOVA.

in different tissues and cells (Stover *et al.* 1987, Lin *et al.* 1993, Cotroneo *et al.* 2001, Fritz *et al.* 2002, Taylor *et al.* 2006) but, to our knowledge, we are the first to show that E₂ regulates cardiac AR receptor mRNA. Pressure-overload hypertrophy is associated with upregulation of the AR in both rats and humans (Thum & Borlak 2002) and E₂ reduces pressure overload-induced hypertrophy (van Eickels *et al.* 2001). Therefore, one may speculate that E₂ may reduce pressure overload-induced hypertrophy via AR downregulation, but this has to be addressed in future studies.

Interestingly, left-ventricular wall thickness has been shown to increase early after menopause, independently of the presence or absence of high blood pressure, resulting in a concentric left-ventricular geometric pattern (Schillaci *et al.* 1998). At menopause, E₂ levels are reduced while testosterone levels are kept fairly constant (Snyder 2001). Thus, according to our findings, the fact that the cardiac effects of androgens are left unopposed by oestrogens constitutes a possible mechanism for the concentric remodelling occurring after menopause. To date there are no large prospective studies of the effect of hormone-replacement therapy on cardiac mass or remodelling (Hayward *et al.* 2001). Given the data on concentric remodelling as an independent predictor of cardiovascular risk (Verdecchia *et al.* 1995) these studies are important to perform. Future studies should also explore the potential utility of anti-androgen therapy in postmenopausal women with cardiac concentric remodelling and hypertrophy.

In the present study E₂ had some effects that were present only in the background of DHT treatment (e.g. the effect on left-ventricular wall thickness, IGF-I mRNA and calculated left-ventricular mass in the DHT-treated rats) and some effects that were independent of concomitant DHT treatment (e.g. the effects on body weight, uterine weight, heart weight and body weight-adjusted cardiac output). The fact that E₂ reduced unadjusted heart weight but increased heart weight adjusted for body weight illustrates the difficulty of evaluating changes in cardiac weight during concomitant changes of body composition. The effect of E₂ to increase body weight-adjusted cardiac output is in line with previous observations (Pelzer *et al.* 2005) and, as blood pressure was unchanged, this suggests that E₂ primarily reduced systemic vascular resistance (Beyer *et al.* 2001), although effects of E₂ on cardiac contractility have also been suggested (Pelzer *et al.* 2005).

The dose of DHT used in this study was selected according to previous dose-response studies in orchidectomized male rats, and we chose a dose that resulted in prostate weight and seminal vesicle weight of the same magnitude as seen in sham-operated rats (Vandenput *et al.* 2002). We found that DHT treatment increased serum DHT to levels approximately 2.6-fold higher than those of sham rats and the selected dose of E₂ resulted in both a higher uterus weight and higher serum levels of E₂ in

E₂-treated compared with sham rats. Thus, the cardiac effects seen in this study were achieved by treatment with supraphysiological doses of both E₂ and DHT. However, it should be noted that the DHT treatment were given in a background of very low androgen levels as ovariectomy *per se* reduced serum concentrations of testosterone by 94%.

In conclusion, treatment with the endogenous non-aromatizable androgen DHT causes cardiac concentric remodelling in OVX rats, possibly mediated by increased local levels of IGF-I. The effects of DHT on cardiac wall thickness and IGF-I mRNA were antagonized by E₂. These mechanisms may be of importance for the concentric left-ventricular geometric pattern developing in women after menopause.

Acknowledgements

The authors are grateful to Katrien Venken and Professor Dirk Vanderschueren, Laboratory for Experimental Medicine and Endocrinology, Katholieke Universiteit Leuven, Leuven, Belgium, for generously providing silastic implants and to Britt-Mari Larsson, Elin Silverpil and Gunnel Andersson for excellent assistance.

Funding

The study was supported by the Swedish Heart and Lung Foundation, the Novo Nordisk Foundation, the Göteborg Medical Society, the Tore Nilson Foundation, the Emelle Foundation, the Swedish Medical Society and the Swedish Research Council (12206). The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

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Received in final form 13 February 2006

Accepted 17 March 2006

Made available online as an Accepted Preprint
22 March 2006