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# Elevated mean arterial pressure in the ovariectomized rat was normalized by $ET_A$ receptor antagonist therapy: absence of cardiac hypertrophy and fibrosis

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**1** The influence of menopause on ventricular function and remodelling remains undefined. The following study examined the effect of ovariectomy on ventricular contractility, cardiac hypertrophy and extracellular matrix protein expression.

**2** Elevated circulating levels of the vasoconstrictor endothelin-1 have been reported in post-menopausal women. Moreover, endothelin-1 has been shown to influence blood pressure, ventricular function and cardiac remodelling. In this regard, the potential pathophysiological role of endothelin-1 in the ovariectomized rat was assessed *via* the administration of the selective $endothelin_A$ receptor ($ET_A$) antagonist BMS-182874.

**3** In 3 and 6 week ovariectomized female Sprague–Dawley rats, uterus atrophy was associated with a significant increase in mean arterial pressure, and left ventricular systolic pressure, as compared to sham. By contrast, right ventricular contractile indices were normal in the ovariectomized rat. Despite increased systolic load, left ventricular hypertrophy was not evident, prepro-atrial natriuretic peptide (prepro-ANP) mRNA levels and collagen protein content were similar to sham.

**4** The treatment of ovariectomized rats with BMS-182874 (60 mg $kg^{-1}$ per day) did not reverse uterus atrophy. However, BMS-182874 normalized mean arterial pressure, and left ventricular systolic pressure in the ovariectomized rat.

**5** Thus, despite elevated blood pressure, ovariectomized rats were not associated with either cardiac hypertrophy or fibrosis. Lastly, endothelin-1, acting *via* the stimulation of the $ET_A$ receptor represents an integral mechanism implicated in the increase of mean arterial pressure following ovariectomy.

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**Keywords:** Ovariectomy; hypertension; cardiac hypertrophy; fibrosis; endothelin-1

**Abbreviations:** BW, body weight; $ET_A$, $endothelin_A$ receptor; $E_2$, Oestradiol; (ER-$\alpha$), oestrogen receptor-$\alpha$; (ER-$\beta$), oestrogen receptor-$\beta$; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; LV, left ventricle; LVEDP, left ventricular end-diastolic pressure; LVSP, left ventricular systolic pressure; MAP, mean arterial pressure; (prepro-ANP), prepro-atrial natriuretic peptide; RV, right ventricle; RVEDP, right ventricular end-diastolic pressure; RVSP, right ventricular systolic pressure

## Introduction

It has been well established that the incidence of cardiovascular disease in pre-menopausal women is lower than age-matched men (Schwartz *et al*., 1995). Albeit, in post-menopausal women, this trend was lost, and found to be quantitatively similar to men (Becker, 1995; Schwartz *et al*., 1995). Coronary artery disease, as well as cerebrovascular stroke represent two salient complications associated with menopause, and may be in part related to the increase in circulating LDL, oxidized-LDL, and concomitant decrease in HDL production (Simon *et al*., 2001; Greendale *et al*., 1999; Matthews *et al*., 1989). Lastly, an increased incidence of systemic hypertension has been documented in post-menopausal women (Reckeloff, 2001), and identified as an independent risk factor in the development of coronary artery disease, cerebrovascular stroke, as well as congestive heart failure (Stokes *et al*., 1989; Kannel *et al*., 1976). Consequently, it has been proposed that ovarian hormones represent an intrinsic cardioprotective mechanism and in this regard, hormonal replacement therapy in post-menopausal women has received considerable interest.

The increased production of nitric oxide represents the primary mechanism by which oestrogen influences vascular tone. Acting *via* genomic and non-genomic pathways, oestrogen treatment of endothelial cells stimulated nitric oxide synthase mRNA transcription and enzyme activity, respectively (Kim *et al*., 1999; Kleinert *et al*., 1998). Nitric oxide is a potent vasodilator of the underlying vasculature, and an important antiproliferative factor (Lloyd-Jones & Bloch, 1996; Garg & Hassid, 1989). Secondly, oestrogen treatment of endothelial cells increased the synthesis of the vasodilator prostacyclin *via* a non-genomic mechanism (Jun

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*et al*., 1998). Thirdly, *in vivo* studies have demonstrated that oestrogen inhibited adverse vascular smooth cell remodelling (Chen *et al*., 1996). Concomitant with these latter effects, the treatment of endothelial cells with oestrogen, as well as progesterone abrogated the expression of the vasoconstrictor/proliferative peptide endothelin-1 (Morey *et al*., 1998). Consistent with this latter finding, endothelin-1 plasma levels were reported increased in post-menopausal women (Wilcox *et al*., 1997). Collectively, these direct and indirect actions of ovarian hormones on the vasculature may at least in part explain the lower incidence of cardiovascular disease in pre-menopausal women.

Despite the plethora of data regarding the pathophysiological events linked to the development of vascular disease in post-menopausal women, very little is known with regard to potential maladaptive effects of menopause on ventricular contractility and cardiac remodelling. Secondly, although hormonal replacement therapy represents a rational approach to reduce the incidence of cardiovascular disease, important deleterious secondary events have been described. These latter effects include the increased production of the atherogenic marker C-reactive peptide, increased incidence of thromboembolism, gall bladder disease and breast cancer (Cushman *et al*., 1999; Ridker *et al*., 1998; Hulley *et al*., 1998; Steinberg *et al*., 1991; Dupont & Page, 1991). Consequently, alternative therapeutic approaches ameliorating cardiovascular function without the deleterious secondary effects associated with hormonal replacement therapy would be advantageous. Based on this latter observation, it is possible that elevated plasma endothelin-1 levels documented in post-menopausal women may contribute to the increased incidence of cardiovascular disease, and therefore represent a potential pharmacological target. Thus, the following study examined whether ovariectomy adversely affected ventricular function, cardiac hypertrophy, and extracellular matrix remodelling. Second, the potential role endothelin-1 in cardiac function and remodelling in the ovariectomized female rat was assessed *via* the administration of the selective $ET_A$ receptor antagonist BMS-182874.

## Methods

### *Ovariectomy*

Female Sprague–Dawley rats (9–11 weeks; Charles River, St. Constant, Quebec, Canada) were anaesthetized with a ketamine (50 mg $kg^{-1}$)/xylazine (10 mg $kg^{-1}$) mixture and underwent either a sham surgery or bilateral ovariectomy, and sacrificed 3 or 6 weeks later. Following haemodynamic measurements, the uterus (uterine horn), and the heart (right and left ventricles were separated) were excised, immediately weighed, frozen in liquid nitrogen, and stored at $-80^{\circ}C$. Left ventricular weight included the free wall of the left ventricle (LV) and the septum. In parallel experiments, the aryl sulphonamide, selective non-peptidic orally active $ET_A$ receptor antagonist BMS-182874 (60 mg $kg^{-1}$ per day; Bristol Myers Squibb, Canada) was added to rat chow 1 week following ovariectomy and continued for 2 weeks (Battistini & Dussault, 1998). BMS-182874 has a $\approx$1000 fold higher affinity for $ET_A$ versus $ET_B$ and doses of 40 to 100 mg $kg^{-1}$ per day have been used to examine the role of the $ET_A$ receptor in hypertension, and vascular remodelling (Park & Schiffrin, 2001; Rossi *et al*., 2000; Battistini & Dussault, 1998; Ferrer *et al*., 1995). In a second series of experiments, oestradiol ($E_2$; 0.1 mg kg $day^{-1}$) was injected IP immediately following ovariectomy, and continued for 3 weeks. The use and care of laboratory animals was according to the Canadian Council for Animal Care and approved by the Animal Care Committee of the Montreal Heart Institute.

### *Haemodynamic measurements*

Rats were anaesthetized with a ketamine (50 mg $kg^{-1}$)/xylazine (10 mg $kg^{-1}$) mixture. A microtip pressure transducer catheter (model SPR-407, 2F; Millar Instrument, Houston, TX, U.S.A.) was inserted into the right carotid artery to obtain arterial blood pressure and heart rate tracings. The catheter was subsequently advanced into the LV to measure systolic and end-diastolic pressures. The left ventricular maximum rate of contraction ($+dp/dt$) and relaxation ($-dp/dt$) were derived by active analogue differentiation of the pressure signal. The Millar catheter was subsequently inserted into the right ventricle (RV) *via* the right jugular vein for the measurement of right ventricular contractile indices. Haemodynamic measurements were recorded on a Gould 2600-second recorder. Following the haemodynamic measurements, the heart and uterus were excised and frozen immediately in liquid nitrogen, and subsequently stored at $-80^{\circ}C$.

### *Plasma endothelin-1 levels*

Following haemodynamic measurements, arterial blood was withdrawn, and endothelin-1 levels were measured by an enzyme immunoassay, as indicated by the manufacturer (Biomedica Gruppe, Austria).

### *Northern hybridization*

Total RNA from the left ventricular wall (not including the septum) was isolated by a modification of guanidine thiocyanate-phenol-chloroform extraction method, as previously described (Nguyen *et al*., 2000). Total RNA (20 $\mu$g) was denatured with formaldehyde and formamide, and separated by size electrophoresis on a 1.3% agarose/4% formaldehyde gel, and subsequently transferred to nylon membranes (GeneScreen Plus; Dupont-NEN) by vacuum blotting (model 785; Bio-Rad Laboratories). A 0.7 kb fragment of rat prepro-atrial natriuretic peptide (courtesy of Dr M. Boluyt), and a 1.2 kb fragment of rat glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (American Type Culture Collection; Rockville, MD, U.S.A.) were labelled with [$^{32}$P]-dCTP (NEN) to a specific activity of $1-2\times10^6$ c.p.m. per ng cDNA by the random hexamer (Pharmacia) priming method and hybridized to nylon membranes (Dupont-NEN) for 18–24 h at 42°C, as previously described (Nguyen *et al*., 2000). The filters exposed to the cDNA probes were washed twice (15 min, room temperature) with 300 mmol $l^{-1}$ NaCl/30 mmol $l^{-1}$ trisodium citrate and 0.1% SDS and twice (15 min, 45°C) with 30 mmol $l^{-1}$ NaCl/3 mmol $l^{-1}$ trisodium citrate and 0.1% SDS. Nylon membranes were subsequently exposed to Kodak XAR film with an intensifying screen at $-70^{\circ}C$, and films were scanned with

a laser densitometer (Chemilmager 4000 I v4.04 software; Alphan Innotech corporation). All levels of mRNA reported in this paper are normalized to the level of GAPDH mRNA.

### *Assessment of collagen*

Collagen type-1, and type-3 content in the LV were quantified in cross sections cut into 8 $\mu$m thick slices, and stained with Sirius Red F3BA as a 0.1% solution in saturated aqueous picric acid, as previously described (Pickering *et al*., 1996). Histological sections were viewed at 400 × magnification with an Olympus microscope (Carson Group Inc, Markam, Ontario, Canada) connected to a Sony colour video monitor. Six randomly selected fields in the endocardium, and 10 in the epicardium from sham ($n=4$) and 6 week ovariectomized rats ($n=4$) were analysed. Image analysis was performed with the software Scion Image 1.6 (Scion, Co) on a Power Macintosh computer. The collagen volume density per cent was calculated as the sum of all collagen points in each field divided by the area of the field.

### *Western blot analysis*

Left ventricular tissue or the uterus was homogenized in a buffer containing (in mM): TRIS 10 (pH.7.5), NaCl 150, EDTA 1, EGTA 1, NaF 50, $\beta$-glycerophosphate 20, phenylmethylsulphonyl flouride 0.5, sodium vanadate 1, 1% Triton X-100, 0.5% nonidet P-40 and 1 $\mu$g ml$^{-1}$ of leupeptin and aprotinin. The subsequent ventricular or uterus lysate was centrifuged for 5 min, the supernatant frozen and stored at $-80^{\circ}$C. BioRad assay was used to determine protein content, and 300 $\mu$g of either ventricular or uterus lysate was subjected to SDS-polyacrylamide gel (10%) electrophoresis, and subsequently transferred to Hybond-C membrane (Amersham Canada Limited). The membrane was pre-incubated in 10 mM TRIS pH 7.4, 150 mM NaCl and 0.1% Tween (v v$^{-1}$) (TBS-T buffer) containing 3% skim milk for 1 h at room temperature, and subsequently incubated overnight at 4°C with either 1 $\mu$g ml$^{-1}$ of a rabbit-polyclonal antibody directed against oestrogen receptor-$\alpha$ (ER-$\alpha$), or a goat-polyclonal antibody directed oestrogen receptor-$\beta$ (ER-$\beta$) (Santa Cruz Biotechnology). Following overnight incubation, the membrane was washed three times with TBS-T containing 3% skim milk, and subsequently incubated for 1 h at room temperature with the appropriate secondary antibody (1 : 10,000) conjugated to horseradish peroxidase (Santa Cruz Biotechnology). Following incubation, the membrane was washed three times with TBS-T and the bands were detected by autoradiography utilizing the ECL detection kit (Amersham Canada Limited). Films were scanned with a laser densitometer (Chemilmager 4000 v4.04 software; Alphan Innotech corporation).

### *Statistics*

All data are presented as the mean ± s.e.mean. Morphological, and haemodynamic measurements were evaluated by a 2 way ANOVA and significant difference was determined by the Neuman–Keuls test. A $P$ value $<0.05$ was considered as statistically significant. Statistical analysis of endothelin-1 plasma levels, oestrogen receptor expression, collagen content, and prepro-ANP mRNA expression between sham and ovariectomized rats was performed by a unpaired two tailed student $t$-test, and a $P$ value $<0.05$ was considered as significant.

## Results

### *Morphometric measurements and plasma endothelin-1 levels in the ovariectomized rat*

Three weeks following ovariectomy, body weight (BW) was significantly increased, whereas absolute uterus weight was significantly decreased, as compared to sham (Table 1). LV/BW, and RV/BW ratios were similar between sham and 3 week ovariectomized rats (Table 1). In 6 week ovariectomized rats, a further increase in BW and decrease in absolute uterus weight was observed, as compared to 3 week ovariectomized rats (Table 2). LV/BW and RV/BW ratios in the 6 week ovariectomized rats were significantly lower, as compared to sham (Table 2). However, this latter observation was most likely due to the increase in BW gain. Lastly, in 3 week ovariectomized rats, plasma endothelin-1 levels were modestly increased ($1.47 \pm 0.12$ fmoles ml$^{-1}$; $n=8$), as compared to sham ($1.29 \pm 0.09$ fmoles ml$^{-1}$; $n=7$), but was not statistically significant.

### *Haemodynamic measurements of the ovariectomized rat*

In 3 week ovariectomized rats, mean arterial pressure (MAP), and left ventricular systolic pressure (LVSP) were significantly increased (Table 3), and remained elevated in 6 week ovariectomized rats, as compared to sham (Table 4). The rate of left ventricular contraction (+dp/dt) and relaxation (−dp/dt) were increased in both the 3 and 6 week ovariectomized rats, albeit only −dp/dt was statistically significant, as compared to sham (Tables 3 and 4). The increase in dp/dt indices may reflect enhanced ventricular contractility in the ovariectomized rat, or represent an adaptive change secondary to the increased systolic load. When +dp/dt was corrected for LVSP (dp/dt/LVSP), a modest non-significant decrease was observed in the 3 week ovariectomized rat (Table 3), whereas a significant decrease was evident 6 weeks following ovariectomy (Table 4). By contrast, when −dp/dt was corrected for LVSP, no difference was observed between sham and ovariectomized rats at either 3 or 6 weeks (Tables 3 and 4). Left ventricular end-diastolic pressure (LVEDP) was significantly increased in 3 week ovariectomized rats, as compared to sham (Table 3). In 6 week ovariectomized rats, LVEDP remained elevated, but it did not reach statistical significance (Table 4). Lastly, heart rate and right ventricular contractile indices were similar between sham and ovariectomized rats at both 3 (data not shown) and 6 weeks (Table 4).

### *Prepro-ANP mRNA expression and collagen content in the LV of ovariectomized rats*

Putative features of cardiac remodelling associated with increased systolic load include myocyte hypertrophy, fibroblast proliferation and the concomitant increase in the synthesis and secretion of extracellular matrix proteins. The significant increase in BW observed in the ovariectomized rats may have potentially masked the presence of a

**Table 1** Morphometric measurements of the 3 week ovariectomized rat and the effect of BMS-182874 and $E_2$

| | *BW* (g) | *LV* (g) | *RV* (g) | *LV*/*BW* $\times 10^3$ | *RV*/*BW* $\times 10^3$ | *Uterus* (g) | *Uterus*/*BW* $\times 10^3$ |
|---|---|---|---|---|---|---|---|
| Sham ($n=11$) | 260±3 | 0.53±0.01 | 0.16±0.004 | 2.05±0.048 | 0.62±0.01 | 0.41±0.02 | 1.58±0.08 |
| OVX ($n=10$) | 323±4[a] | 0.63±0.02[a] | 0.19±0.005[a] | 1.91±0.040 | 0.58±0.01 | 0.17±0.02[a] | 0.52±0.02[a] |
| Sham + $E_2$ ($n=6$) | 256±7 | 0.55±0.02 | 0.14±0.01[a] | 2.13±0.050 | 0.53±0.04 | 0.47±0.02 | 1.75±0.06 |
| OVX + $E_2$ ($n=6$) | 242±3[b] | 0.56±0.01[b] | 0.13±0.01[b] | 2.30±0.04[b] | 0.54±0.06 | 0.39±0.04[b] | 1.61±0.15[b] |
| Sham + BMS ($n=4$) | 278±4 | 0.55±0.02 | 0.15±0.006 | 1.98±0.04 | 0.55±0.03 | 0.58±0.04[a] | 2.10±0.02[a] |
| OVX + BMS ($n=8$) | 322±8[a] | 0.67±0.01[a] | 0.18±0.003[a] | 2.09±0.05 | 0.56±0.01 | 0.15±0.01[a] | 0.46±0.02[a] |

OVX denotes ovariectomized rats; BMS, BMS-182874 (60 mg kg $day^{-1}$); $E_2$ oestradiol (0.1 mg kg $day^{-1}$); BW, body weight; LV, left ventricle; RV, right ventricle; data are presented as mean±s.e.mean; [a]Represents $P<0.05$ versus Sham; [b]Represents $P<0.05$ versus OVX and ($n$) denotes number of rats in each group.

**Table 2** Morphometric measurements of the 6 week ovariectomized rat

| | *BW* (g) | *LV* (g) | *RV* (g) | *LV*/*BW* $\times 10^3$ | *RV*/*BW* $\times 10^3$ | *Uterus* (g) | *Uterus*/*BW* $\times 10^3$ |
|---|---|---|---|---|---|---|---|
| Sham ($n=7$) | 287±12 | 0.57±0.02 | 0.15±0.005 | 2.00±0.083 | 0.54±0.03 | 0.42±0.02 | 1.49±0.11 |
| OVX ($n=8$) | 356±7[a] | 0.64±0.02[a] | 0.18±0.003[a] | 1.80±0.044[a] | 0.48±0.03[a] | 0.10±0.01[a] | 0.29±0.02[a] |

OVX denotes ovariectomized rats; BW, body weight; LV, left ventricle; RV, right ventricle; data are presented as mean±s.e.mean; [a]Represents $P<0.05$ versus Sham and ($n$) denotes number of rats in each group.

**Table 3** Haemodynamic parameters of the 3 week ovariectomized rat and the effect of BMS-182874 and $E_2$

| | *LVSP* (mmHg) | *LVEDP* (mmHg) | *LV+dP/dt* (mmHg/s) | *LV-dP/dt* (mmHg/s) | *+dp/dt/LVSP* ($s^{-1}$) | *dp/dt/LVSP* ($s^{-1}$) | *MAP* (mmHg) |
|---|---|---|---|---|---|---|---|
| Sham ($n=9$) | 115±4 | 7±1 | 6200±243 | 5066±321 | 54±1.3 | 44±1.6 | 106±4 |
| OVX ($n=9$) | 142±8[a] | 13±2[a] | 6777±315 | 6088±284[a] | 48±2.3 | 43±1.7 | 122±5[a] |
| Sham + $E_2$ ($n=6$) | 99±6 | 5±1 | 4458±567 | 4125±500 | 44±3.0 | 42±3.0 | 90±10 |
| OVX + $E_2$ ($n=6$) | 95±6[b] | 4±0.5[b] | 4916±684[b] | 4125±626[b] | 44±7.0 | 37±5.0 | 93±8[b] |
| Sham + BMS ($n=4$) | 118±6 | 7±1 | 5069±420 | 5070±357 | 55±3.0 | 43±2.5 | 106±1 |
| OVX + BMS ($n=9$) | 116±5[b] | 13±1[a] | 5139±120[b] | 5139±219[b] | 55±3.0 | 45±1.7 | 109±3[b] |

OVX denotes ovariectomized rats; BMS, BMS-182874 (60 mg kg $day^{-1}$); $E_2$ oestradiol (0.1 mg kg $day^{-1}$); LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; +dP/dt, rate of contraction; −dP/dt, rate of relaxation; MAP, mean arterial pressure; data are presented as mean±s.e.mean; [a]Represents $P<0.05$ versus Sham; [b]Represents $P<0.05$ versus OVX and ($n$) denotes number of rats in each group.

**Table 4** Morphometric measurements of the 6 week ovariectomized rat

| | *LVSP* (mmHg) | *LVEDP* (mmHg) | *LV+dP/dt* (mmHg/s) | *LV-dP/dt* (mmHg/s) | *+dp/dt/LVSP* ($s^{-1}$) | *dp/dt/LVSP* ($s^{-1}$) | *RSVP* (mmHg) | *RVEDP* (mmHg) | *MAP* (mmHg) | *Heart rate* (beats/min) |
|---|---|---|---|---|---|---|---|---|---|---|
| Sham ($n=7$) | 108±3 | 5±1 | 6257±264 | 5422±143 | 58±1.9 | 52±1.5 | 24±1 | 2±0.2 | 97±4 | 252±9 |
| OVX ($n=8$) | 140±7[a] | 9±2 | 6937±290 | 6593±21[a] | 51±0.7[a] | 48±1.0 | 25±1 | 3±0.6 | 120±5[a] | 253±11 |

OVX denotes ovariectomized rats; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; RSVP, right ventricular systolic pressure; RVEDP, right ventricular end-diastolic pressure; +dP/dt, rate of contraction; −dP/dt, rate of relaxation; MAP, mean arterial pressure; data are presented as mean±s.e.mean; [a]Represents $P<0.05$ versus Sham and ($n$) denotes number of rats in each group.

hypertrophic response. In this regard, the steady-state mRNA level of prepro-ANP, a marker of cardiac myocyte hypertrophy was examined in the LV of ovariectomized rats. In 3 week ovariectomized rats, a modest decrease (27±33%↓ vs sham; $n=4$) of prepro-ANP mRNA expression was observed. In the 6 week ovariectomized rats, prepro-ANP mRNA levels were unchanged (18±8%↓ vs sham; $n=4$) in the LV, as compared to sham (Figure 1). Lastly, a putative feature of cardiac fibrosis is the increased synthesis of the extracellular matrix protein collagen. In the epicardium, and endocardium of the LV of 6 week ovariectomized rats, collagen type-1 and type-3 protein content were not significantly different from sham (Table 5 and Figure 2).

### *The pattern of oestrogen receptor expression in the uterus and LV of ovariectomized rats*

In lysates isolated from the normal female rat uterus ($n=6$), the ER-α subtype was highly expressed in the uterus, whereas the ER-β was undetectable (Figure 3). In lysates isolated from the LV of a normal female rat, the ER-α and ER-β subtypes were both expressed ($n=4$) (Figure 3). However, as compared to the uterus, the electrophoretic mobility of the ER-α was slower in the LV. In the 3 week ovariectomized rat ($n=5$), oestrogen receptor expression in the LV was similar to sham (Figure 3). By contrast, the electrophoretic mobility of the ER-α was slower in the uterus of ovariectomized rats

**Table 5** Collagen content in the LV of 6 week ovariectomized rats

| | *Epi Collagen Type 1 (%)* | *Epi Collagen Type 3 (%)* | *End Collagen Type 1 (%)* | *End Collagen Type 3 (%)* |
|---|---|---|---|---|
| Sham ($n=4$) | 0.035±0.003 | 0.0034±0.0004 | 0.027±0.002 | 0.0031±0.0005 |
| OVX ($n=4$) | 0.035±0.003 | 0.0033±0.0001 | 0.026±0.003 | 0.0022±0.0005 |

OVX denotes 6 week ovariectomized rats; Epi, epicardium (10 randomly selected fields of each rat examined); End, endocardium (six randomly selected fields of each rat examined) of the LV; data are presented as mean±s.e.mean, and ($n$) denotes number of rats in each group. The collagen volume density per cent was calculated as the sum of all collagen points in each field divided by the area of the field.

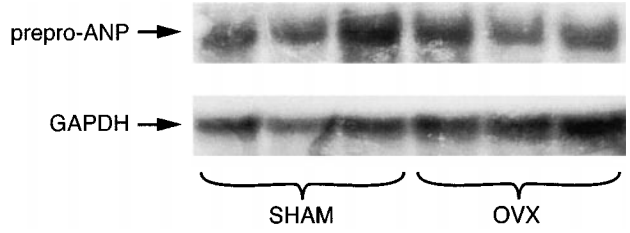


**Figure 1** The pattern of prepro-ANP mRNA expression in the LV of ovariectomized rats. In 6 week ovariectomized rats, prepro-ANP mRNA level in the LV was unchanged, as compared to sham (see Results). Prepro-ANP mRNA levels were normalized to GAPDH mRNA.

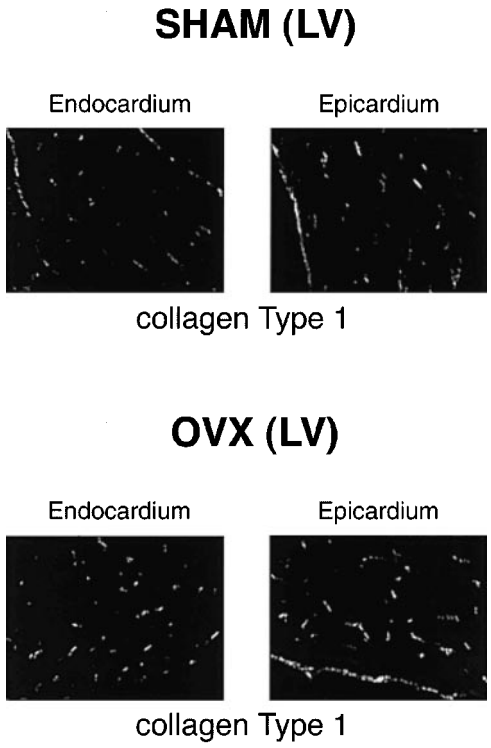


**Figure 2** Collagen content in the LV of 6 week ovariectomized rats. Sirius red staining of the LV of ovariectomized rats demonstrated that the level of collagen type-1 protein expression in the endocardium and epicardium was similar to sham (see Table 3).

($n=6$), as compared to sham (Figure 3). Moreover, this latter electrophoretic pattern of the uterine ER-$\alpha$ in the ovariectomized rat was analogous to that observed in the LV of the sham rat (Figure 3). In some but not all ovariectomized rats, the faster migrating band of the ER-$\alpha$ observed in the normal uterus was present, albeit, its level of expression in the ovariectomized rat was significantly less than normal (data not shown).

### *The effect of $ET_A$ receptor antagonist BMS-182874 on morphology and the haemodynamic profile of the ovariectomized rat*

Although plasma endothelin-1 levels were not significantly increased in the 3 week ovariectomized rats, this peptide is primarily secreted abluminally, and thus acts as a local autocrine/paracrine factor (Wagner *et al*., 1992). In this regard, the potential contribution of locally released endothelin-1 in the maintenance of elevated MAP in ovariectomized rats was examined *via* the administration of the selective $ET_A$ receptor antagonist BMS-182874. The treatment of sham rats for a period of 2 weeks with BMS-182874 had no effect on cardiac morphology, BW gain, heart rate, or haemodynamic indices (Tables 1 and 3). However, a significant increase in uterus weight was observed with BMS-182784, as compared to sham (Table 1). The administration of BMS-182874 1 week following ovariectomy and continued for 2 weeks normalized elevated MAP and LVSP (Table 3). Although, LV +dp/dt was non-significantly increased in the ovariectomized rat, the treatment with BMS-182874 normalized this parameter (Table 3). Likewise, BMS-182874 treatment normalized elevated LV −dp/dt in the ovariectomized rat (Table 3). In addition, the modest reduction of +dp/dt/LVSP in the ovariectomized rat was reversed following the administration of BMS-182874. By contrast, LVEDP in the ovariectomized rats remained elevated following BMS-182874 therapy (Table 3).

### *The effect of $E_2$ on morphology and the haemodynamic profile of the ovariectomized rat*

The administration of $E_2$ to sham rats for 3 weeks had no significant effect on BW, uterus weight, LV/BW, or RV/BW ratios (Table 1). Moreover, $E_2$ treatment did not significantly alter MAP, or contractile indices (Table 3). In the ovariectomized rat, $E_2$ administration prevented the increase in BW, and uterus atrophy (Table 1). Secondly, $E_2$ therapy prevented the increase in MAP, LVSP, and LVEDP in the 3 week ovariectomized rat (Table 3). Although, left ventricular +dp/dt was non-significantly increased in the ovariectomized rat, this contractile parameter was similar to sham following $E_2$ treatment (Table 3). Likewise, $E_2$ administration normalized elevated left ventricular −dp/dt in the ovariectomized rat (Table 3).

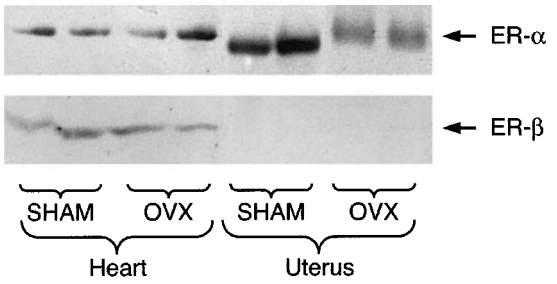


**Figure 3** Oestrogen receptor subtype expression in the heart and uterus and the subsequent effect of ovariectomy. The ER-α (~68 kDa) and -β subtypes (~59 kDa) were expressed in the LV of a normal female rat. Following ovariectomy (3 weeks), oestrogen receptor expression was unchanged. In the uterus of a normal female rat, the ER-α was expressed, whereas the ER-β was undetected. Interestingly, the electrophoretic mobility of the ER-α subtype in the LV was slower, as compared to the uterus. Following ovariectomy, the electrophoretic mobility of ER-α was shifted upward in the atrophied uterus, and was analogous to that observed in the LV of either a sham or ovariectomized rat.

## Discussion

It has been clearly established that the incidence of cardiovascular disease is increased in post-menopausal women. However, very little is known with regard to any potential maladaptive effect of menopause on cardiac function and remodelling. To examine this latter premise, bilateral ovariectomy was performed on female Sprague–Dawley rats, and within 3 weeks a significant increase in MAP, and LVSP was observed, and remained elevated 6 weeks post-ovariectomy. The rate of left ventricular contraction and relaxation were increased in the ovariectomized, albeit only $-dp/dt$ was found to be significantly different from sham. When dp/dt indices were corrected for LVSP, a small but significant decrease in $+dp/dt/LVSP$ was observed in the ovariectomized rat (6 weeks), suggestive of a modest impairment of systolic function. Indeed, in the isolated working heart, contractile performance was diminished in the ovariectomized rat, and was shown to be due in part to an increase in $V_3$ myosin heavy chain expression, and a reciprocal decrease in the $V_1$ isoform expression (Scheuer *et al*., 1987). Left ventricular end-diastolic pressure was significantly increased in 3 week ovariectomized rats, and remained elevated 6 weeks post-ovariectomy. By contrast, right ventricular contractile indices in the ovariectomized rat were similar to sham. Moreover, despite elevated MAP in the ovariectomized rat, neither cardiac hypertrophy nor fibrosis was evident.

The lower incidence of cardiovascular disease in pre-menopausal women has been attributed primarily to the ovarian hormone oestrogen. Several studies have demonstrated that oestrogen stimulated the synthesis and release of the vasodilator/antiproliferative factor nitric oxide *via* both a genomic and non-genomic mechanism acting at the level of nitric oxide synthase (Kim *et al*., 1999; Kleinert *et al*., 1998). Secondly, oestrogen increased the synthesis of the potent vasodilator prostacylin *via* a non-genomic action in endothelial cells (Jun *et al*., 1998). Concomitant with the increased synthesis of vasodilatory factors, oestrogen treatment of endothelial cells inhibited the expression of the potent vasoconstrictor/proliferative peptide endothelin-1 (Morey *et al*., 1998). Consequently, the decrease in circulating oestrogen following menopause would favour a vasoconstrictor dominance of vascular tone. Indeed, circulating plasma endothelin-1 levels were reported higher in post-menopausal women, as compared to pre-menopausal women (Wilcox *et al*., 1997). Moreover, endothelin-1, acting *via* the $ET_A$ receptor has been implicated in the development of both systemic and pulmonary hypertension (Schiffrin, 1998; Prie *et al*., 1997; Dicarlo *et al*., 1995). In this regard, plasma endothelin-1 levels were measured, and a modest increase was observed in 3 week ovariectomized rats, as compared to sham, but was not statistically significant. This observation was not unexpected since endothelin-1 is predominantly released abluminally from endothelial cells, and thus acts primarily as an autocrine/paracrine factor (Wagner *et al*., 1992). Consistent with this latter premise, plasma endothelin-1 levels were normal in the DOCA-salt hypertensive rat model, albeit the administration of a non-selective ET receptor decreased elevated blood pressure, and ameliorated vascular remodelling (Calderone *et al*., 1994; Li *et al*., 1994). In this regard, the potential paracrine role of locally released endothelin-1 on the underlying vasculature of the ovariectomized rat was examined. The administration of the selective $ET_A$ receptor antagonist BMS-182874 1 week post-ovariectomy and continued for 2 weeks normalized MAP, LVSP, dp/dt indices, and dp/dt corrected for LVSP, without any secondary effect on either BW gain or uterus atrophy. Collectively, these data suggest that $ET_A$ receptor antagonist therapy can suppress the increase in blood pressure following ovariectomy. It remains to be determined whether either an increase in local endothelin-1 synthesis and/or reactivity of the $ET_A$ receptor was the underlying mechanism. By contrast, despite the normalization of MAP and LVSP, LVEDP in the ovariectomized rat remained elevated following $ET_A$ receptor antagonist therapy. Consequently, the modulation of LVEDP in the ovariectomized rat was apparently independent of MAP, left ventricular contractility, or endothelin-1 activation of the $ET_A$ receptor. Instead, the increase of LVEDP may be a consequence of decreased levels of circulating ovarian hormones. Consistent with this concept, the administration of estradiol prevented the increase in LVEDP, as well as LVSP, and MAP. Although the underlying mechanism by which $E_2$ prevented the increase of LVEDP remains undetermined, a role for nitric oxide may be postulated. In the myocardium, nitric oxide has been shown to increase diastolic distensibility (Prendergast *et al*., 1997; Paulus *et al*., 1994), and nitric oxide synthase activity in the heart can be enhanced by oestrogen treatment (Neudling *et al*., 1999)

A sustained increase in systolic load, as observed in systemic hypertension leads to a concentric pattern of cardiac hypertrophy, characterized by the increased expression of the putative hypertrophic marker prepro-ANP mRNA and the progression of interstitial fibrosis (Boluyt & Bing, 1995; Calderone *et al*., 1995; Weber & Brilla, 1991; Grossman *et al*., 1975). Direct morphological examination was not performed to determine whether a concentric pattern of cardiac remodelling had occurred. However, LV/BW ratio was similar between sham and ovariectomized rats, suggesting the absence of a hypertrophic response. To further confirm this latter premise, left ventricular prepro-ANP mRNA expression was examined. The steady-state mRNA

level of prepro-ANP was unchanged in the LV of the 3 and 6 week ovariectomized rats, as compared to sham. Lastly, in 6 week ovariectomized rats, the percentage of interstitial collagen in the epicardium, and endocardium of the LV was similar to sham. Collectively, these data underscore the absence of classical cardiac remodelling (e.g. hypertrophy and fibrosis) in the ovariectomized rat, despite the presence of increased systolic load. It is possible that the increase in MAP documented in the ovariectomized rat may not have been sufficient to promote either cardiac hypertrophy or fibrosis. Alternatively, an intrinsic difference between the male and female rat heart may exist, and thus selectively influence the pattern of cardiac remodelling. In fact, a recent study by Weinberg *et al.* (1999) demonstrated significant gender differences in cardiac remodelling and ventricular function, despite a similar degree of left ventricular hypertrophy and systolic wall stress.

In the left ventricle of a normal female rat, ER-$\alpha$, and ER-$\beta$ subtypes were expressed, whereas only the ER-$\alpha$ subtype was detected in the uterus. Following ovariectomy, the expression of both oestrogen receptor subtypes in the LV was unchanged, as compared to sham. By contrast, in the atrophied uterus of the ovariectomized rat, a distinct upward electrophoretic shift of the ER-$\alpha$ was evident, and displayed a similar mobility to that observed in the LV. These data highlight a disparate pattern of oestrogen receptor subtype expression and mobility following ovariectomy between the heart and uterus. Collectively, these findings support the premise that ventricular oestrogen receptor expression was not influenced by endogenous changes in circulating oestrogen.

In summary, elevated MAP represents an underlying feature of ovariectomy, albeit neither cardiac hypertrophy nor fibrosis was evident. The treatment of ovariectomized rats with the selective $ET_A$ receptor antagonist BMS-182874 normalized MAP, LVSP, and dp/dt indices, without any secondary effect on BW gain or uterus atrophy. Despite the rationale that hormonal replacement therapy can reduce the risk of cardiovascular disease in post-menopausal women, numerous detrimental secondary effects have been documented. Consequently, the use of $ET_A$ receptor antagonist therapy could potentially represent a pharmacological approach to treat hypertension in post-menopausal women.

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