British Journal of Pharmacology (2000) 130, 1092–1098

www.nature.com/bjp

Deoxycorticosterone acetate-salt hypertensive rats display gender-related differences in ET_B receptor-mediated vascular responses

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1 Male DOCA-salt rats exhibit vasoconstriction upon ET_B activation. Because hypertension is less severe in female than male DOCA rats, we hypothesized that female DOCA rats would display attenuated ET_B vasoconstrictor responses.

2 Uninephrectomized Wistar rats received DOCA and drinking water containing NaCl/KCl. Control rats received vehicle and tap water. Systolic blood pressure was higher in male *vs* female DOCA rats. Responses to endothelin-1 (ET-1), IRL-1620, an ET_B agonist, and acetylcholine were evaluated in isolated aortas and *in vivo* in the mesenteric microcirculation.

3 Endothelium-denuded aortas from male, but not female, DOCA rats displayed increased sensitivity to ET-1. IRL-1620 contracted aortas from male DOCA rats, but not control or female DOCA aortas. Noradrenaline-constricted and endothelium-intact aortas from male, but not female, DOCA rats displayed increased relaxation to IRL-1620 compared to control aortas.

4 *In vivo*, increased vasoconstriction to ET-1 was observed in male and female DOCA rats. IRL-1620 induced vasodilation in control rats, but vasoconstriction in male DOCA rats. There were minimal changes in diameter in vessels from female DOCA rats.

5 The initial fall in blood pressure induced by ET-1 and IRL-1620 was attenuated in male DOCA rats. Bosentan, a mixed ET_A/ET_B receptor antagonist, lowered blood pressure in male and female DOCA rats, but a greater and marked decrease occurred in the male DOCA group.

6 The gender-related differences in ET-1/ET_B-mediated effects both in the vasculature and blood pressure suggest that sex-related functional up-regulation of ET_B receptors may play a role in the more severe hypertension in male DOCA hypertensive rats.

British Journal of Pharmacology (2000) **130,** 1092–1098

Keywords: DOCA-salt hypertension; endothelin; gender; ET_B receptors; IRL-1620

Abbreviations: ACh, acetylcholine; DOCA-salt rats, deoxycorticosterone-salt hypertensive rats; ET-1, endothelin-1; ET_A receptor, ET_A receptor subtype; ET_B receptor, ET_B receptor subtype; IRL-1620, Suc-[Glu9,Ala11,15]-endothelin-1(8-21), a selective ET_B agonist; MABP, mean arterial blood pressure; NA, noradrenaline; SBP, systolic blood pressure; SNP, sodium nitroprusside

Introduction

Endothelin-1 (ET-1) is a potent vasoconstrictor peptide produced by endothelial and vascular smooth muscle cells and its effects are mediated by activation of both ET_A and ET_B receptor subtypes. Both receptors stimulate phosphatidylinositol hydrolysis and arachidonic acid release in response to agonist stimulation, although different G proteins are activated and differential regulation of cyclic AMP transduction cascades are observed. ET_A receptors are widely expressed in both vascular and nonvascular smooth muscles whereas ET_B receptors are expressed predominantly on endothelial cells and, to a much lesser extent, on vascular smooth muscle cells (Kanaide, 1996; Schiffrin & Touyz, 1998). Based on their cellular location, it was suggested that vascular smooth muscle cells ET_A receptors mediate vasoconstriction and endothelial ET_B receptors elicit vasodilation, by the release of prostanoids and endothelium-derived relaxant factors (EDRFs) (Kanaide, 1996). However, it has been shown that both ET_A and ET_B receptors mediate contraction in various vascular territories, such as dog coronary arteries (Cannan *et al*., 1996), rat renal vasculature (Matsuura *et al*., 1996), rabbit portal vein (Wang *et al*., 1997a), and rat mesenteric arteries and veins (Mickley *et al*., 1997; Johnson *et al*., 1998). It has also been demonstrated that ET_A and ET_B receptors may undergo both functional and molecular up- or down-regulation in certain physiopathological conditions such as arterial hypertension (Nguyen *et al*., 1992; Barber *et al*., 1996; Cannan *et al*., 1996; Wang *et al*., 1997b; Ivy *et al*., 1998).

Several studies in animal models of hypertension, including DOCA-salt hypertensive rats, have shown that females do not develop hypertension as quickly or as severely as males (Cambotti *et al*., 1984; Iams & Wexler, 1977; Ouchi *et al*., 1987; Lange *et al*., 1998). The mechanisms involved in this gender-related difference have not been completely elucidated. Considering that ET-1 plays a role in the development of DOCA-salt hypertension (Nguyen *et al*., 1992; Deng & Schiffrin, 1992; Larivière *et al*., 1993a,b; Li *et al*., 1994; Matsumura *et al*., 1999) and that ET-1- and ET_B-mediated vasoconstriction is increased in DOCA-salt hypertensive rats (Carvalho *et al*., 1990; Zhao *et al*., 1998; Johnson *et al*., 1998), we hypothesized that arteries from male DOCA-salt rats would display increased ET_B-mediated vasoconstrictor responses compared with female DOCA-salt rats. We evaluated vascular reactivity to ET-1 and to IRL-1620, an ET_B selective

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agonist (Takai *et al*., 1992), in male and female DOCA-salt hypertensive rats by using isolated endothelium-intact and -denuded aortic rings. We further extended our investigation to resistance vessels from the mesenteric microcirculation and experiments were carried out *in situ* and *in vivo* to evaluate if similar alterations would be observed in other vascular beds. We also evaluated the effects of ET-1, IRL-1620 and Bosentan, a mixed ET_A/ET_B receptor antagonist (Clozel *et al*., 1994) on blood pressure of male and female control and DOCA-salt hypertensive rats.

Methods

DOCA-salt induced hypertension

Male and female 8-week-old Wistar rats underwent uninephrectomy (small flank incision, left side) during chloral hydrate anaesthesia (300 mg kg^{-1}, i.p.). One week after surgery, rats received subcutaneous injection of DOCA (30–50 mg kg^{-1} $week^{-1}$) and drinking water supplemented with 1.0% NaCl and 0.2% KCl. Control rats received vehicle injections and normal tap drinking water. All animals were fed standard laboratory rat chow, had *ad libitum* access to both food and water and were housed individually in a room with constant temperature (24°C) with a 12-h dark–light cycle. Systolic blood pressure (SBP) was taken weekly by the standard tail-cuff method (pneumatic transducer, PowerLab 4/S, AD Instruments Pty Ltd., Australia) in conscious restrained rats, before and after surgeries. Experimental protocols included in this manuscript followed standards and policies of the University of Sao Paulo's Animal Care and Use Committee.

Vascular reactivity in isolated aortic rings and mesenteric microvessels in vivo/in situ

At 6 weeks after surgery rats were anaesthetized with chloral hydrate (400 mg kg^{-1}, sc.), killed by an induced pneumothorax and experiments were performed in thoracic aortic rings from control and DOCA-hypertensive groups, as previously described (Carvalho *et al*., 1990). Concentration-response curves to ET-1, IRL-1620, a selective ET_B agonist, ACh and SNP were evaluated in endothelium-intact or endothelium-denuded aortic rings. The responses to ET-1 and IRL-1620 were also evaluated in microvessels (first order arterioles, 15–20 μm) from the mesenteric microcirculation *in vivo*/*in situ* by using intravital microscopy (Carvalho *et al*., 1990). Briefly, the mesentery was exteriorized and maintained on a special board with a transparent plate for transillumination in the microscopy stage. The mesentery was constantly bathed with Ringer–Locke solution containing 1% gelatin (37°C, pH 7.3) and first order arterioles were selected (capillaries excluded). Each section of the vascular bed was tested only once and no more than four concentrations of the agonist were tested in a single rat. The images ($\times$3400 enlargement) were sent to a 500-line television camera adapted to the microscope phototube and recorded. Changes in vessel diameter are expressed as percentage changes from the initial resting diameter.

Arterial blood pressure measurements

Male and female rats from the control and DOCA-salt hypertensive groups were anaesthetized with chloral hydrate (300 mg kg^{-1}, i.p.) and catheters were placed in the abdominal aorta (to record systemic arterial blood pressure and heart rate) and vena cava (for intravenous bolus injections of ET-1, IRL-1620 and Bosentan) *via* the left femoral artery and vein. All tubing was tunnelled under the skin and exited at the midscapular region. Tubing was filled with saline (0.9% NaCl solution) containing heparin (25 U.I. ml^{-1}) to prevent obstructive thrombus formation. After completion of the surgery, animals were housed in individual cages under a 12-h light–dark cycle with access to standard laboratory chow and drinking water *ad libitum*. Animals were allowed at least 48 h to recover from surgical intervention before mean arterial blood pressure (MABP) was registered. On the day of experimentation, basal MABP was registered during 60 min, by using a strain gauge BP transducer (Statham P23ID; Oxnard, CA, U.S.A.) and a Gould recorder (5900 Signal Conditioner, Gould Instruments Inc, Ohio, U.S.A.). After stabilization animals received a bolus i.v. dose of Bosentan (10 mg kg^{-1}) and MABP was registered during 180 min. At the end of the protocol, responses to 100 pmoles kg^{-1} ET-1 were tested to evaluate the effectiveness of blockade by Bosentan. Other groups of control and DOCA-salt rats received i.v. bolus injection of saline and responses to ET-1 (100 pmoles kg^{-1}) and IRL-1620 (1 nmol kg^{-1}) were also evaluated.

Drugs

ET-1, IRL-1620 and BQ-788 were purchased from Calbiochem-Novabiochem (San Diego, CA, U.S.A.) and deoxycorticosterone acetate, chloral hydrate, noradrenaline bitartrate, sodium nitroprusside and acetylcholine chloride were purchased from Sigma Chemical Co. (St Louis Missouri, U.S.A.). Bosentan was kindly provided by Dr Martine Clozel (Actelion Ltd., Allschwil, Switzerland).

Drugs were prepared daily and dissolved in distilled water and kept on ice during the experiments. Peptides were dissolved in oxygen-free deionized water (degassed under reduced pressure with nitrogen) and stock solutions were aliquoted and frozen.

Data analysis and statistical evaluation

At least six animals were included in each experimental group. Values are expressed as mean $\pm$ s.e.mean. Vasodilation induced by ACh and IRL-1620 are expressed as percentage of inhibition of NA-induced contraction. EC_{50} (concentration of agonist producing 50% of maximal contraction or relaxation), EC_{20} and maximal or minimal responses were estimated by linear regression analysis (fitted to the Hill equation) from log concentration-response curves and expressed as $-\log EC_{50}$ (pD_2 values), $-\log EC_{20}$ and per cent of maximal response. Statistical evaluation of the data was carried out by two-way ANOVA followed by the multiple comparison Bonferroni *t*-test (SigmaStat, version 2.0, Jandel Scientific Software). $P<0.05$ was considered statistically significant.

Results

Male DOCA rats developed an earlier and more severe form of hypertension than female DOCA rats ($P<0.05$). At 2 and 4 weeks of DOCA-salt treatment, SBP was: male, 177 ± 8 and 203 ± 6 mmHg ($n=24$); female, 145 ± 6 and 168 ± 6 mmHg, ($n=25$), respectively. In the control groups, on the day of experimentation, SBP was 123 ± 2 mmHg in male rats ($n=27$), and 119 ± 3 mmHg in female rats ($n=29$). These values did not differ from those at the beginning of treatment. Male DOCA-

salt rats had significantly higher body weights than female rats and weight gain was reduced in the DOCA-salt groups compared to sham or control groups (results not shown). Due to the difference in body weight between male and female rats, water (water + 1% NaCl/0.2% KCl) intake was evaluated as millilitres per 100 grams body weight. Female DOCA-salt rats showed a tendency to consume more water than male DOCA-salt rats, but the difference did not reach statistical significance. At 2 and 4 weeks of DOCA-salt treatment, fluid intake was: male, 0.38 ± 0.05 and 0.54 ± 0.06 ml/100 g body weight ($n = 6$); female, 0.41 ± 0.05 and 0.63 ± 0.07 ml/100 g body weight ($n = 6$), respectively.

Gender differences in vascular reactivity – aortic rings

Vessels were contracted with 90 mmol l^{-1} KCl after the equilibration period. Responses of aortic rings to KCl were similar in control (male = 2.8 ± 0.3 *vs* female = 2.9 ± 0.3 g; $n = 14$ and 15, respectively) and in DOCA-salt rats (male = 3.0 ± 0.3 *vs* female = 2.9 ± 0.4 g, $n = 16$ and 17, respectively). Maximum relaxation to ACh was decreased in aortic rings from DOCA-salt rats, both in male (DOCA = $78.5 \pm 5.1\%$ *vs* Control = $99.7 \pm 0.9\%$, maximum relaxation, $n = 8$) and in female (DOCA = $83.2 \pm 2.5\%$ *vs* Control = $97.9 \pm 1.5\%$, maximum relaxation, $n = 8$) compared with respective controls ($P < 0.05$). No differences in SNP-induced vasodilation were observed between arteries from DOCA-salt and control rats, neither in the male (pD_2 and maximal relaxation: DOCA = 7.2 ± 0.1, 100%; Control = 7.3 ± 0.1, 100%, $n = 7$) or in the female groups (DOCA = 7.3 ± 0.1, 100%; Control = 7.4 ± 0.1, 100%, $n = 7$). As shown in Figure 1, no differences in pD_2 values to ET-1 were observed between male DOCA-salt (8.1 ± 0.1, $n = 7$) and control endothelium-intact arteries (8.3 ± 0.1, $n = 7$). Removal of endothelium increased sensitivity to ET-1 ($P < 0.05$), but no differences in pD_2 values between male DOCA (8.9 ± 0.1, $n = 8$) and control arteries (8.7 ± 0.1, $n = 8$) were observed.

However, endothelium-denuded, but not endothelium-intact, vessels from male DOCA-salt rats displayed increased sensitivity (expressed as $-\log EC_{20}$; $P < 0.05$) to ET-1 (DOCA = 10.5 ± 0.5 *vs* Control = 9.2 ± 0.1, $n = 8$) in comparison with male control rats. No changes in pD_2 values (endothelium intact, DOCA = 8.2 ± 0.1 *vs* Control = 8.3 ± 0.1, $n = 7$; endothelium denuded, DOCA = 8.6 ± 0.1 *vs* Control = 8.6 ± 0.2, $n = 8$) or sensitivity ($-\log EC_{20}$, DOCA = 9.2 ± 0.3, Control = 9.1 ± 0.1, $n = 8$) were observed in arteries from female DOCA-salt rats (Figure 1). No differences in maximum responses to ET-1 were observed between DOCA (male = 5.3 ± 0.7; female = 4.9 ± 0.4 g, $n = 8$) and control arteries (male = 4.9 ± 0.4; female = 4.9 ± 0.5 g, $n = 8$).

Since differences in ET-1 vascular reactivity were observed only at the lowest concentrations of ET-1 (EC_{20}) and responses were blocked in the presence of the selective ET_B receptor antagonist BQ-788 ($-\log EC_{20}$; in male DOCA-salt rats after incubation with 10^{-6} M BQ-788 = 9.4 ± 0.3, $n = 6$; $P < 0.05$), we tested the vascular effects of an ET_B-selective receptor agonist, IRL-1620, on aortas from male and female DOCA-salt and control rats (Figure 2). IRL-1620 induced contraction in endothelium-denuded aortas from male DOCA-salt (maximum contraction = 1.8 ± 0.5 g, $n = 8$), but not in endothelium-intact aortas (0.2 ± 0.1 g, $n = 7$). Vessels from female DOCA-salt rats did not exhibit significant contractions to IRL-1620 either in the absence (0.2 ± 0.1 g, $n = 7$) or in the presence (0.1 ± 0.1 g, $n = 8$) of an intact endothelium. No significant changes in tonus were observed in aortas from male (0.1 ± 0.1 g, $n = 8$) or female (0.05 ± 0.05 g, $n = 8$) control rats.

Endothelium-intact aortas, which were previously constricted with noradrenaline, from male DOCA-salt rats displayed increased relaxation to IRL-1620 ($69.3 \pm 3.5\%$, $n = 8$) compared to male control rats ($23.5 \pm 8.3\%$, $n = 8$;

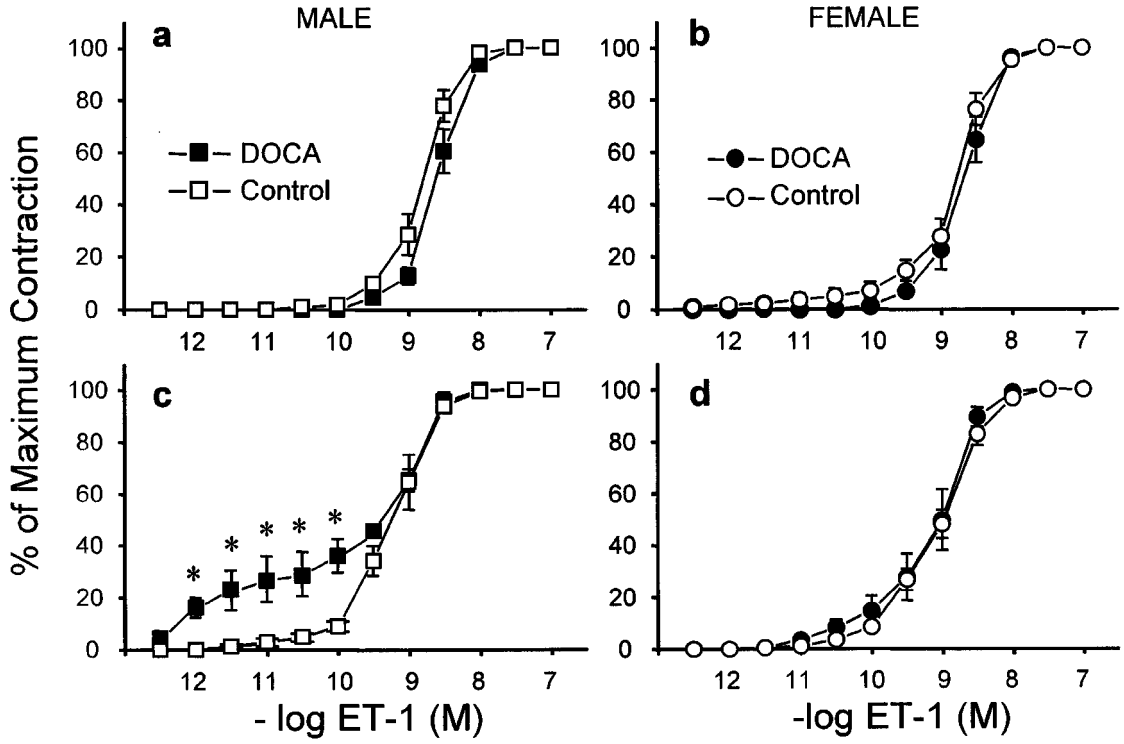

Figure 1 Concentration-response curves to ET-1 in (a,b) endothelium-intact and (c,d) endothelium-denuded aortic rings from male and female control and DOCA-salt hypertensive rats. Data are expressed as percentage of maximal contraction evoked by ET-1. Each point represents mean ± s.e.mean of 7–8 experiments. *$P < 0.05$ *versus* control.

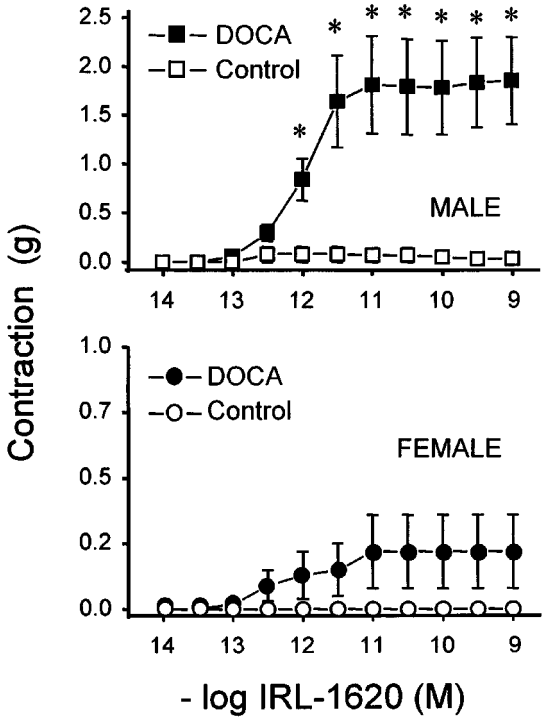

Figure 2 Concentration-response curves to the ET_B selective agonist IRL-1620 in endothelium-denuded aortic rings from male and female control and DOCA-salt hypertensive rats. Data are expressed as grams of contraction evoked by the agonist. Each point represents mean ± s.e.mean of eight experiments. $^*P<0.05$ *versus* control.

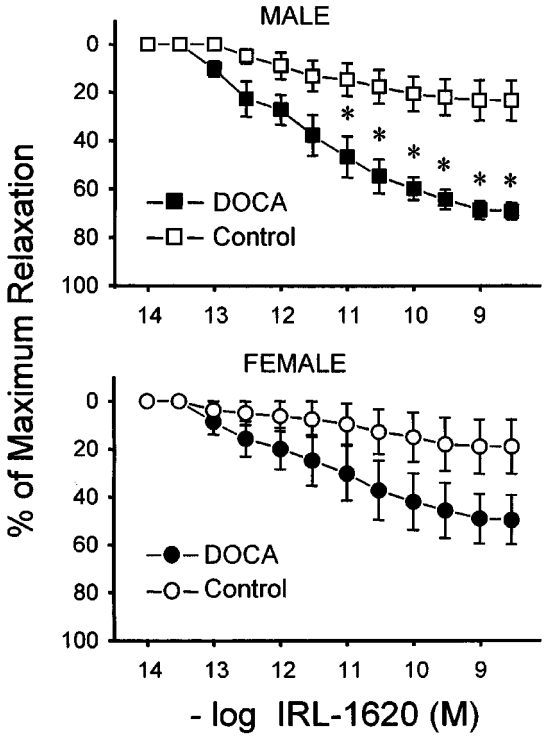

Figure 3 Concentration-response curves to the ET_B selective agonist IRL-1620 in endothelium-intact aortic rings from male and female control and DOCA-salt hypertensive rats. Vessels were stimulated with noradrenaline ($1-3\times10^{-7}$ M) and, when the contraction reached a plateau, cumulative concentrations of IRL-1620 were added to the bath. Data are expressed as percentage of noradrenaline-induced contraction. Each point represents mean ± s.e.mean of eight experiments. $^*P<0.05$ *versus* control.

$P<0.05$) (Figure 3). A tendency to increased IRL-1620-induced relaxation, that did not reach statistical significance, was observed in female DOCA-salt aortas (49.6 ± 10.3%, $n=8$) *vs* female control aortas (19.0 ± 11.2%, $n=8$) (Figure 3).

Gender differences in vascular reactivity – mesenteric microcirculation

In the microcirculation, ET-1 vascular reactivity was increased in male and female DOCA-salt rats in comparison to respective male and female control rats (Figure 4). However, doses 10 times higher were necessary in female rats to produce contractions similar to those observed in male rats. IRL-1620 induced vasodilation in male and female control rats (per cent change initial diameter, male = +8.0 ± 0.8%, $n=8$; female = +6.2 ± 0.6%, $n=10$), but marked vasoconstriction in male DOCA rats (−8.2 ± 1.1%, $n=9$). In the female DOCA group ($n=12$), the rats exhibit both vasodilation and vasoconstriction. In the experiments where vasoconstriction was observed, changes in vessel diameter were not so evident as in the male group, and when vasodilation was observed it was greatly impaired in comparison to that in the female control group (Figure 4). ACh-induced vasodilation was also impaired in vessels from DOCA-salt rats in comparison to control rats. However, as in the aortic vessels, similar values were observed between male and female DOCA-salt rats (per cent change initial diameter; Control ($n=7$), male = +8.5 ± 0.9%, female = +7.8 ± 0.8% and DOCA-salt ($n=8$), male = +6.1 ± 0.9%, female = +5.8 ± 0.7%).

Effects of ET-1, IRL-1620 and bosentan on blood pressure

MABP was higher in DOCA-salt rats compared with control rats ($P<0.05$). On the day of experimentation MABPs were: DOCA-salt, male = 175 ± 3 mmHg, $n=8$; female = 155 ± 8 mmHg, $n=8$ *vs* Control, male = 120 ± 2 mmHg, $n=7$; female = 118 ± 3 mmHg, $n=7$. ET-1 (100 pmoles kg^{-1}) and IRL-1620 (1 nmol kg^{-1}) produced a long and sustained pressor effect preceded by a transient depressor action. The depressor effects of both ET-1 and IRL-1620 were attenuated in the male DOCA-salt hypertensive rats compared to respective control rats. The pressor response to IRL-1620 was greater in male, but not in female, DOCA-salt rats in comparison to control rats (Figure 5). Intravenous bolus injection of bosentan (10 mg kg^{-1}, i.v.) slightly, but significantly, decreased blood pressure in the control groups (after 90 min, male $\Delta = -10 \pm 1$ mmHg, $n=7$ *vs* female $\Delta = -4 \pm 2$ mmHg, $n=7$). Bosentan significantly reduced blood pressure in male and female DOCA-salt hypertensive rats, but the decrease on blood pressure was significantly greater in the male group ($P<0.05$) (Figure 6).

Discussion

The aim of the present study was to evaluate whether altered ET_B receptor-mediated vascular responses are attenuated in female DOCA-salt compared to male DOCA-salt hypertensive rats. Our hypothesis was based on three major observations: (1) in DOCA-salt hypertension BP rises more rapidly and reaches a higher level in male than in female rats (Ouchi *et al.*, 1987; Lange *et al.*, 1998), (2) changes in ET_B vascular reactivity have been observed in vessels from DOCA-salt hypertensive rats (Johnson *et al.*, 1998; Zhao *et al.*, 1998) and (3) vascular functional changes may contribute to the elevated peripheral vascular resistance and blood pressure in DOCA-salt hypertension (Bohr & Webb, 1988).

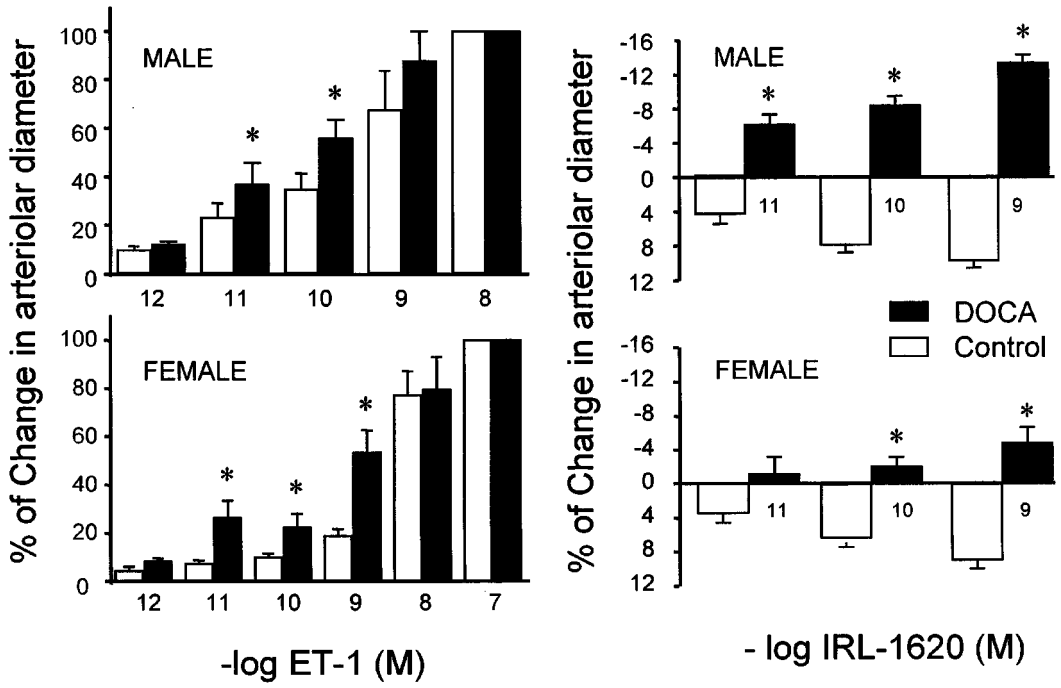

Figure 4 Bar graphs show ET-1-induced contraction and IRL-1620-induced responses, vasoconstriction or vasodilation, in mesenteric microvessels from male and female control and DOCA-salt hypertensive rats. Data are expressed as percentage of change of initial microvessel diameter. Each bar represents mean ± s.e.mean of 8–12 experiments. $*P<0.05$ *versus* respective controls.

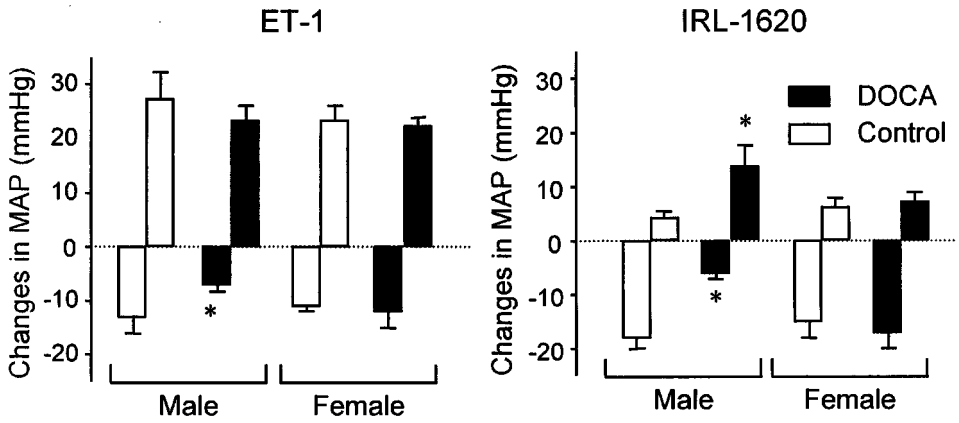

Figure 5 Peak depressor and pressor responses observed following i.v. injection of endothelin-1 (ET-1 100 pmoles kg^{-1}) and IRL-1620 (1 nmol kg^{-1}) in conscious control and DOCA-salt rats. Each bar represents mean ± s.e.mean of six experiments. $*P<0.05$ *versus* respective control.

Experimental evidence indicates that ET-1 plays a role in the pathogenesis of DOCA-salt hypertension: (1) arteries from DOCA-salt hypertensive rats display altered vascular reactivity to ET-1 (Carvalho *et al*., 1990; Nguyen *et al*., 1992; Deng & Schiffrin, 1992; Zhao *et al*., 1998; Johnson *et al*., 1998); (2) ET-1 immunoreactivity is increased in endothelial cells of aorta and mesenteric arteries of DOCA-salt rats (Larivière *et al*., 1993b); (3) ET-1 mRNA is also elevated in vessels of these animals (Larivière *et al*., 1993a); (4) acute administration of ET_A-selective receptor or non-selective ET_A/ET_B receptor antagonists to DOCA-salt rats produces marked hypotensive effects (Doucet *et al*., 1996); (5) chronic administration of ET-1 receptor antagonists attenuates the development of hypertension and reduce vascular hypertrophy and remodelling in DOCA-salt rats (Li *et al*., 1994; Matsumura *et al*., 1999). ET_B-mediated vascular effects have also been shown to be altered in DOCA-salt hypertensive rats. In rat mesenteric veins, sarafotoxin S6c, an ET_B receptor agonist, caused impaired vasodilation in DOCA-salt rats (Johnson *et al*., 1998). In the cremaster arterioles, in the presence of ET_A receptor blockade, ET-1 induces vasodilation in normotensive rats, but significant vasoconstriction was observed in DOCA-salt rats (Zhao *et al*., 1998). These studies were all performed in male rats. The role of ET-1 in the development of hypertension in female DOCA-salt rats is unclear. We demonstrate here that DOCA-salt hypertension is less severe in females than in males, that vascular responses to ET_B receptor activation is greater in male than in female rats, and that blockade of ET-1 effects reduces blood pressure more effectively in male DOCA-salt rats.

The attenuated development of hypertension in female DOCA-salt rats may be related to the modulation exerted by the gonadal hormones on ET-1 effects on the cardiovascular system (Barber *et al*., 1996; Wang *et al*., 1997b; Sudhir *et al*., 1997). Crofton & Share (1997) have observed that estrogen

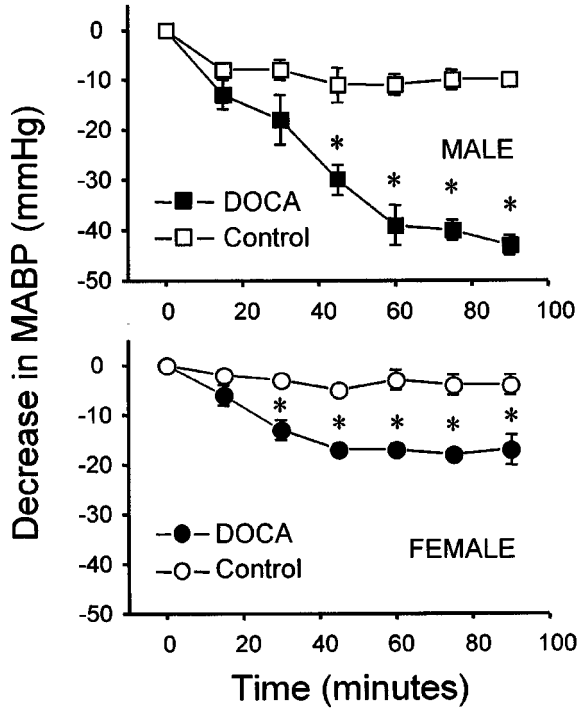

Figure 6 Effects of Bosentan (10 mg kg^{-1} iv.) on mean arterial blood pressure (MABP) of male and female control and DOCA-salt hypertensive rats. Each point represents mean ± s.e.mean of the decrease in basal blood pressure (mmHg) of 7–8 experiments. $^*P<0.05$ *versus* control.

attenuates while testosterone enhances the development of DOCA-salt hypertension. 17β-estradiol attenuates ET-1-induced coronary artery constriction both *in vitro* (Lamping & Nuno, 1996) and *in vivo* (Sudhir *et al*., 1997) and increased expression of prepro-ET-1 mRNA has been observed in porcine aortic endothelial cells in the absence of female ovarian hormones (Wang *et al*., 1997b). In addition, Barber *et al*. (1996) reported that endogenous fluctuations in estrogen influence affinity of endothelin receptor in coronary arterial smooth muscle from female pigs. Gender differences in ET-1 receptor density, as well as in the ratio of ET-1 receptor subtypes, have also been reported (Ergul *et al*., 1998). The authors observed that the total number of ET-1 receptors was increased in human saphenous vein from men in comparison to that in women, while no gender differences in ET-1 binding K_d were observed. It will be very interesting to evaluate if the gender differences in functional up-regulation of ET_B-mediated responses observed in the present study are due to changes in the number/affinity of receptors or in the signalling pathway.

Our results also show that aortas from male, but not female, DOCA-salt rats display increased reactivity to the selective ET_B receptor agonist IRL-1620, only in the absence of the vascular endothelium, indicating the significance of functional ET_B receptors which mediate contraction. In endothelium-intact aortas no changes in IRL-1620 sensitivity were observed, suggesting that endothelial ET_B receptors, which induce vasodilation, modulate the excessive vasoconstriction mediated by activation of vascular smooth muscle ET_B receptors. Indeed, IRL-1620-induced relaxation was increased in aortas from male DOCA-salt compared to aortas from control rats. In aortas from the female DOCA-salt group, where only very mild contractions are seen in response to IRL-1620, the vasodilation was mildly increased. Evaluating the endothelial function with another endothelium-dependent agonist, we observed that male and female DOCA-salt arteries exhibited similar decreases in ACh-induced vasodilation. These results suggest that in DOCA-salt hypertension ET_B receptor-mediated effects are increased in arterial smooth muscle and endothelial cells and that these responses are greater in arteries from male compared to female rats. One may argue that responses seen in isolated and endothelium-denuded vessels may not be representative of vessels *in vivo* since endothelial cells modulate agonist-induced contraction. However, by using intravital microscopy, we observed that IRL-1620 induced vasodilation in mesenteric microvessels from control rats, marked vasoconstriction in male DOCA-salt rats and a very mild vasoconstriction in the female DOCA-salt group. In this experimental condition, where endothelial cells modulate responses to vasoactive agents, we also observed increased vasoconstrictor responses to ET-1 and IRL-1620 in male, but not in female, DOCA-salt hypertensive rats. In this vascular bed, as in the aortic rings, impaired responses to ACh were similar between male and female DOCA-salt rats, suggesting that the gender-related differences in endothelial responses to IRL-1620 are specific. Gender differences in endothelial dysfunction was initially described in aorta from SHR and, in agreement with our findings, endothelial dysfunction was attenuated in vessels from female rats (Kauser & Rubanyi, 1995), suggesting that female sex steroid hormones may influence endothelial function. A differential ratio of ET_A to ET_B receptors in saphenous vein from men and women, favouring vasodilator effects in women, has also been described (Ergul *et al*., 1998) and provides further support to the hypothesis that slower progression and lower incidence of hypertension in female animals may be related to differences in vascular reactivity. Furthermore, the attenuated transient depressor responses to ET-1 and IRL-1620 in male, but not female, DOCA-salt rats associated with a greater decrease in blood pressure in the male rats, upon treatment with an ET_A/ET_B antagonist, suggests that the gender differences in ET-1 pathways do have a physiological role.

In conclusion, the present study has demonstrated that altered vascular ET-1/ET_B-mediated responses occur in male, but not in female, DOCA-salt hypertensive rats and that endothelial modulation of ET-1 responses differs according to the vascular bed. The present data also show gender differences in the blood pressure effects of ET-1, IRL-1620 and an ET_A/ET_B antagonist in DOCA-salt rats, suggesting that differential activation of ET-1 pathways, expressed by a functional up-regulation of ET_B receptors, may play a role in the higher blood pressure levels observed in male DOCA-salt hypertensive rats.

We are most grateful to Dr Martine Clozel (Actelion Ltd., Allschwil, Switzerland) for the kind donation of bosentan. We also thank Dr Lisete C. Michelini for the laboratory facilities in the measurements of blood pressure, and Sonia M.L. Rodrigues and Maria Aparecida Oliveira for excellent technical expertise. These studies were supported by grants from FAPESP (R.C.A. Tostes Passaglia, F.L. David, Z.B. Fortes) and CNPq (R.C.A. Tostes Passaglia, D. Nigro, Z.B. Fortes, M.H. Catelli de Carvalho).

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(Received November 22, 1999
Revised February 14, 2000
Accepted March 23, 2000)