Brazilian Journal of Medical and Biological Research (2002) 35: 1061-1068
ISSN 0100-879X

Gender differences in vascular expression of endothelin and ET_A/ET_B receptors, but not in calcium handling mechanisms, in deoxycorticosterone acetate-salt hypertension

F.L. David[1,3], A.C.I. Montezano[1], N.A. Rebouças[2], D. Nigro[1], Z.B. Fortes[1], M.H.C. Carvalho[1] and R.C.A. Tostes[1]

Departamentos de [1]Farmacologia, and [2]Fisiologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, SP, Brasil
[3]Departamento de Ciências Biológicas e da Saúde, Universidade Federal de Mato Grosso, Pontal do Araguaia, MT, Brasil

Abstract

We determined if the increased vascular responsiveness to endothelin-1 (ET-1) observed in male, but not in female, DOCA-salt rats is associated with differential vascular mRNA expression of ET-1 and/or ET_A/ET_B receptors or with functional differences in Ca^{2+} handling mechanisms by vascular myocytes. Uninephrectomized male and female Wistar rats received DOCA and drinking water containing NaCl/KCl. Control rats received vehicle and tap water. Blood pressure and contractile responses of endothelium-denuded aortic rings to agents which induce Ca^{2+} influx and/or its release from internal stores were measured using standard procedures. Expression of mRNA for ET-1 and ET_A/ET_B receptors was evaluated by RT-PCR after isolation of total cell RNA from both aorta and mesenteric arteries. Systolic blood pressure was higher in male than in female DOCA rats. Contractions induced by Bay K8644 (which activates Ca^{2+} influx through voltage-operated L-type channels), and by caffeine, serotonin or ET-1 in Ca^{2+}-free buffer (which reflect Ca^{2+} release from internal stores) were significantly increased in aortas from male and female DOCA-salt compared to control aortas. DOCA-salt treatment of male, but not female, rats statistically increased vascular mRNA expression of ET-1 and ET_B receptors, but decreased the expression of ET_A receptors. Molecular up-regulation of vascular ET_B receptors, rather than differential changes in smooth muscle Ca^{2+} handling mechanisms, seems to account for the increased vascular reactivity to ET-1/ET_B receptor agonists and higher blood pressure levels observed in male DOCA-salt rats.

Correspondence
R.C.A. Tostes
Departamento de Farmacologia
ICB, USP
Av. Lineu Prestes, 1524
05508-900 São Paulo, SP
Brasil
Fax: +55-11-3091-7322
E-mail: rtostes@usp.br

Presented at the IV International Symposium on Vasoactive Peptides, Belo Horizonte, MG, Brazil, October 19-21, 2001.

Research supported by FAPESP and CNPq.

Received December 7, 2001
Accepted May 3, 2002

Key words
- DOCA-salt hypertension
- Endothelin-1
- Gender
- Endothelin receptors
- Calcium
- Rats

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 ET_A and ET_B receptor subtypes. In the vascular system, ET_A receptors are expressed in smooth muscle cells whereas ET_B receptors are expressed predominantly in endothelial cells and, to a much lesser extent, in smooth muscle cells (1,2). Both ET_A and ET_B receptor subtypes can activate various signaling mechanisms in vascular smooth muscle, including i) G protein-mediated activation of phospholipase C, leading to phosphatidylinositol hydrolysis and formation of inositol trisphosphate (IP_3) and diacylglycerol, ii) increase in cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$), iii) activation of protein kinase C, and iv) changes in intracellular pH via stimulation of the Na^+-H^+ exchanger (2,3). ET-1 typically mediates a biphasic $[Ca^{2+}]_i$ response consisting of a rapid initial transient phase and a sustained plateau phase. The first $[Ca^{2+}]_i$ transient is generated primarily by IP_3-induced mobilization of intracellular Ca^{2+} and, to a lesser extent, by Ca^{2+}-induced Ca^{2+} release. The second $[Ca^{2+}]_i$ phase, which appears to contribute to the sustained ET-1-induced vasoconstriction, depends on external Ca^{2+} and is the result of transmembrane Ca^{2+} influx, mainly through voltage-dependent L-type Ca^{2+} channels (VOC), which may be directly or indirectly activated by ET-1 (3-5).

We have recently shown that male, but not female, deoxycorticosterone acetate (DOCA)-salt rats exhibit increased vascular responsiveness to ET-1 and IRL-1620, a selective ET_B receptor agonist, and we speculated that up-regulation of ET_B receptors or changes in the signaling pathways could be involved in this response (6). Since male DOCA-salt rats exhibit increased vascular ET-1 expression (7,8), changes in the ratio of ET_A/ET_B receptors (9,10) and altered Ca^{2+} control mechanisms, such as increased transmembrane flux of Ca^{2+} and greater Ca^{2+} mobilization from internal stores (11,12), in the present study we determined if increased vasoconstriction in response to ET-1/IRL-1620 in male, but not female, DOCA-salt rats is associated with differential vascular expression of mRNA for ET-1 and ET_A/ET_B receptors or with functional differences in Ca^{2+} handling mechanisms by vascular myocytes.

Material and Methods

DOCA-salt-induced hypertension and vascular reactivity in isolated vessels

DOCA-salt hypertension was induced in male and female 8-week-old Wistar rats as previously described (6). Systolic blood pressure (SBP) was measured by the standard tail-cuff method (PowerLab 4/S, ADInstruments Pty Ltd., Castle Hill, Australia) in conscious restrained rats. Endothelium-denuded aortas from control and DOCA-salt rats were used to evaluate vascular reactivity to caffeine (5-30 mM), which releases Ca^{2+} from intracellular stores by an IP_3-independent mechanism (13) and to Bay K8644 (10^{-10} to 3×10^{-5} M), which activates Ca^{2+} influx through VOC (14). Functional assessment of Ca^{2+} uptake into the sarcoplasmic reticulum (SR) and caffeine- or agonist-induced intracellular Ca^{2+} mobilization were also evaluated as described elsewhere (12). Briefly, the arteries were stimulated with norepinephrine (3 μM) and allowed to reach a plateau. Ca^{2+}-free buffer was then introduced into the muscle bath for 15 min. After this depletion period, 1.6 mM Ca^{2+} buffer was placed in the muscle bath for 15 min (loading period). Ca^{2+}-free buffer was then reintroduced into the bath and allowed to equilibrate for 1 min before 20 mM caffeine was added. Transient contractions in Ca^{2+}-free buffer upon agonist stimulation, an indirect measurement of IP_3-dependent Ca^{2+} release from the SR, were also elicited by both ET-1 (10 nM) and serotonin (5-HT, 3 μM).

The experimental protocols used in the present study followed the standards and policies of the Committee on Animal Care and Use of the University of São Paulo.

Reverse transcriptase-polymerase chain reaction

Total cell RNA was isolated from aorta and mesenteric arteries using Trizol Reagent (Gibco BRL, Life Technologies, Rockville, MD, USA). After DNA digestion (RQ1 DNAse RNAse-free, Promega Corporation, Madison, WI, USA), total RNA (20 ng per sample) was used for reverse transcriptase (RT) in the presence of an RNase inhibitor (RnasIn®, Promega Corporation), 200 U of Moloney murine leukemia virus RT (Gibco BRL) and 1 µg of oligo (dT)12-18 primer at 37ºC for 60 min, according to manufacturer specifications. The cDNA products were isolated by phenol-chloroform extraction, precipitated with ethanol, resuspended in 120 µl TE (10 mM Tris-HCl and 1 mM EDTA, pH 7.5) and stored at -20ºC until required for the polymerase chain reaction (PCR). PCR primers were designed on the basis of published rat cDNA sequences for glyceraldehyde 3-phosphate dehydrogenase (GAPDH), ET-1, and ET_A/ET_B receptors, and are as follows: ET-1, antisense primer CTCGCTCT ATGTAAGTCATGG, sense primer GCTC CTGCTCCTCCTTGATG, which should amplify a 471-bp fragment; ET_A, antisense primer CTGTGCTGCTCGCCCTTGTA, sense primer GAAGTCGTCCGTGGGCA TCA (216-bp fragment); ET_B, antisense primer CACGATGAGGACAATGAGAT, sense primer TTACAAGACAGCCAAA GACT (565-bp fragment); GAPDH, antisense primer CACCACCCTGTTGCTGTA, sense primer TATGATGACATCAAGAA GGTGG (219-bp fragment). GAPDH was used as an internal control for the co-amplification. In order to identify the optimal amplification conditions, a series of pilot studies were performed using a thermal cycler with a temperature gradient in the annealing step (Eppendorf Mastercycler gradient, Eppendorf-Netheler-Hinz, Hamburg, Germany), various amounts of RT products from 2 to 200 ng RNA, and 20-35 cycles of PCR amplification. The following conditions were selected for PCR in a volume of 50 µl: RT products from 20 ng of RNA, 2.5 U Taq polymerase (Gibco BRL), 28 cycles of amplification for ET-1, 25 cycles for ET_A and ET_B receptor genes, and 20 cycles for the GAPDH gene. Amplification was carried out using an initial denaturing cycle at 94ºC for 5 min and the subsequent cycles as follows: denaturation, 30 s at 94ºC; annealing, 30 s at 55ºC (ET-1, ET_B) or 60ºC (ET_A, GAPDH), and extension, 45 s at 72ºC. PCR products (10 µl per lane) were electrophoresed using 1% agarose gel containing ethidium bromide (0.5 µg/ml). The gel was subjected to ultraviolet light and photographed. The band intensities were measured using a software package (Kodak Digital Science, Eastman Kodak Company, New Haven, CT, USA) and the signals are reported relative to the intensity of GAPDH amplification in each co-amplified sample.

Drugs

DOCA, chloral hydrate, 5-hydroxytryptamine (serotonin), and caffeine were purchased from Sigma (St. Louis, MO, USA), and Bay K8644 was from Calbiochem Corporation (La Jolla, CA, USA).

Data analysis and statistical evaluation

Values are reported as means ± SEM. EC_{50} (i.e., the concentration of agonist producing 50% of maximal contraction) and maximal 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), and percent of maximal response, respectively. Data were analyzed statistically by two-way

ANOVA followed by the multiple comparison Bonferroni test (SigmaStat®, version 2.0, Jandel Scientific Software), with the level of significance set at $P<0.05$.

Results

At 6 weeks of DOCA-salt treatment, systolic blood pressure was significantly elevated in both male and female rats relative to their respective time-matched controls (males 192 ± 6 *vs* 121 ± 4 mmHg, N = 18-20; females 165 ± 8 *vs* 119 ± 3 mmHg, N = 16). Systolic blood pressure was higher in male than in female DOCA-salt-treated rats.

Gender differences in Ca^{2+} handling mechanisms

After the equilibration period, vessels were initially contracted with 90 mM KCl. Responses of aortic rings to 90 mM KCl were similar in control (males 2.6 ± 0.4 g *vs* females 2.8 ± 0.3 g, N = 8) and DOCA-salt rats (males 2.9 ± 0.4 g *vs* females 2.7 ± 0.5 g, N = 8). Figure 1 illustrates the vascular responses to Bay K8644 and caffeine. Aortas from both male and female DOCA-salt rats showed significantly greater contractions to Bay K8644 than control aortas ($P<0.05$). Maximum responses, expressed as percentages of KCl-induced contraction, were: control, males 31 ± 6% *vs* females 25 ± 5% (N = 8) and DOCA-salt, males 100 ± 9% *vs* females 97 ± 6% (N = 9 and 8, respectively). Caffeine-induced contractions were also similarly enhanced in aortas from male and female DOCA-salt rats in comparison to their respective controls ($P<0.05$; maximum responses: control, males 38 ± 3% *vs* females 32 ± 2%, N = 10; DOCA-salt, males 63 ± 5% *vs* females 53 ± 4%, N = 9 and 10, respectively).

Figure 2 illustrates typical records of functional experiments performed to evaluate vascular Ca^{2+} uptake and its release from intracellular SR stores induced by both a

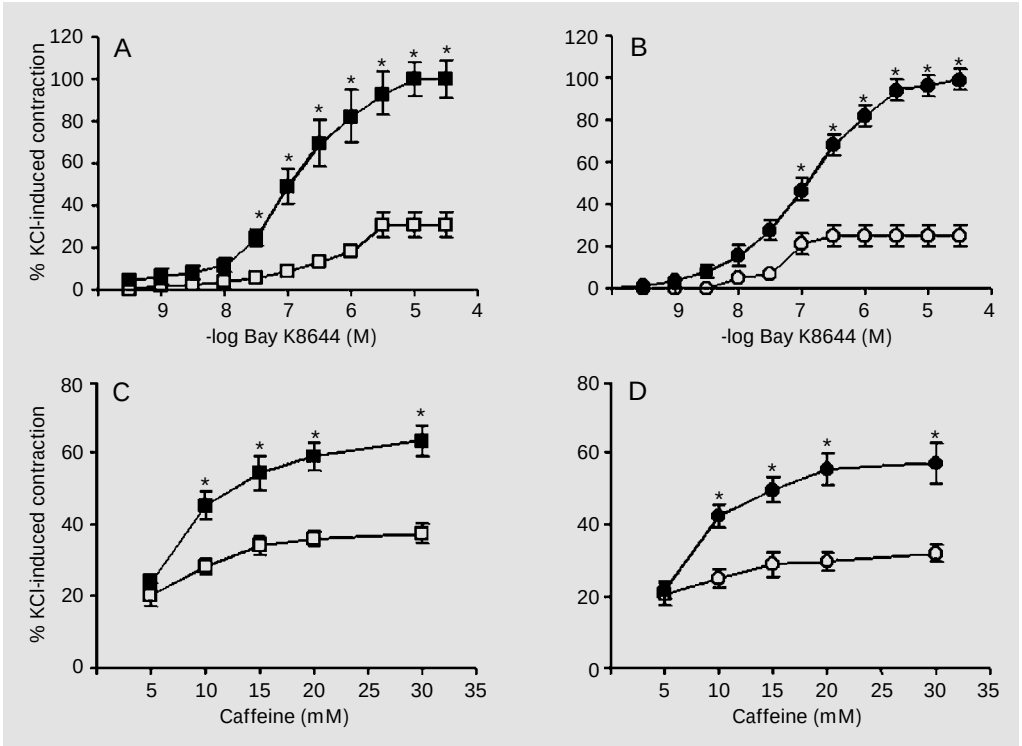

Figure 1. Concentration-response curves to Bay K8644 and to caffeine in endothelium-denuded aortic rings from male (A,C) and female (B,D) control (open circles, open squares) and DOCA-salt hypertensive (filled squares, filled circles) rats. Data are reported as percent of the contraction evoked by 90 mM KCl. Each point indicates the mean ± SEM of 8-10 experiments. *$P<0.05$ *vs* control (two-way ANOVA).

caffeine/IP_3-independent pathway and by an agonist/IP_3-dependent pathway. After initial stimulation with norepinephrine (3 µM), vessels were washed in Ca^{2+}-free buffer for 15 min in order to deplete intracellular Ca^{2+} stores. Vessels were subsequently exposed to Ca^{2+}-containing buffer for 15 min to reload intracellular Ca^{2+} stores and then stimulated with ET-1 (10 nM), 5-HT (3 µM) or caffeine (20 mM) after a 1-min exposure to Ca^{2+}-free buffer. Under these conditions, agonist-induced contraction is an indirect measurement of the buffering capacity of the SR and also reflects agonist-induced intracellular Ca^{2+} mobilization. Arteries from male DOCA-salt rats exhibited spontaneous contractions during the loading period (6.4 ± 2.4% change from basal tonus), which were not observed in control arteries. Aortas from male DOCA-salt rats also exhibited greater contractions in response to ET-1 in comparison to control arteries (51 ± 4 *vs* 9 ± 3%, N = 11 and 10; $P<0.05$). Similar alterations were observed in aortas from female DOCA-salt rats, which displayed a 7.6 ± 3.5% change in basal tonus and greater contractions to ET-1 in comparison to their respective controls (40 ± 4 *vs* 8 ± 3%, N = 8; $P<0.05$). Likewise, preparations from both male and female DOCA-salt-treated rats displayed greater contractions to both 5-HT and caffeine when compared to those from their respective time-matched control groups (5-HT: males 34 ± 4 *vs* 19 ± 3%, females 39 ± 3 *vs* 18 ± 3%, N = 10-11; caffeine: males 31 ± 5 *vs* 11 ± 2%, females 32 ± 5 *vs* 12 ± 3%, N = 6-8; $P<0.05$ for each comparison).

Gender differences in ET-1 and ET_A/ET_B receptor mRNA expression

The results obtained by RT-PCR showing expression of mRNA for ET-1, ET_A and ET_B receptors in aortas and mesenteric arteries from male and female control and DOCA-salt rats are shown in Figure 3. DOCA-salt treatment significantly increased ET-1 mRNA expression both in the aorta and mesenteric arteries from male rats, while no significant changes were observed in the same vessels from female rats. A tendency to decreased ET_A receptor mRNA expression was observed in the aorta and in the mesenteric artery in the male DOCA-salt group ($P = 0.052$ and $P = 0.06$, respectively), while no changes were observed in the female DOCA-salt group. A significant increase in the mRNA expression of the ET_B receptor subtype was observed in both arteries from male DOCA-salt rats, but not in female DOCA-salt vessels.

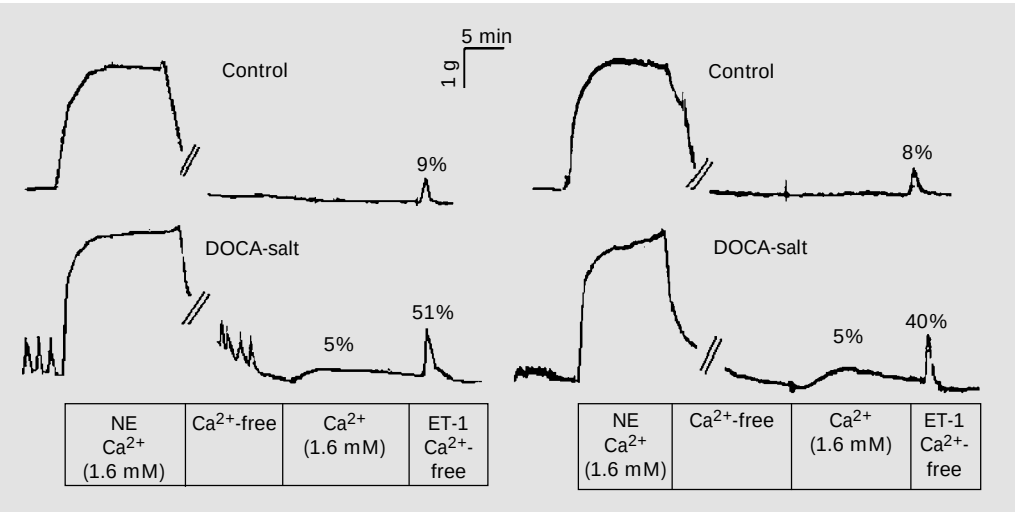

Figure 2. Ca^{2+} uptake into the sarcoplasmic reticulum and agonist-induced Ca^{2+} release. Aortas from male (left) and female (right) control and deoxycorticosterone acetate (DOCA)-salt hypertensive rats were stimulated with norepinephrine (NE, 3 µM) and allowed to reach a plateau. Ca^{2+}-free buffer was then introduced into the muscle bath for 15 min. After this depletion period, 1.6 mM Ca^{2+} buffer was placed in the muscle bath for 15 min (loading period). Ca^{2+}-free buffer was then reintroduced into the bath and allowed to equilibrate for 1 min before an agonist (10 nM endothelin) was added. All responses are reported as percent of the initial response to KCl in 1.6 mM Ca^{2+}. Tracings are representative of 8-11 experiments.

Discussion

We have demonstrated that male DOCA-salt rats display increased vascular sensitivity to ET-1 and to IRL-1620, an ET_B receptor agonist which induces contraction in aortas from male DOCA-salt rats, but not in preparations from control or female DOCA-salt animals (6). These gender-related differences in ET-1/IRL-1620 vascular reactivity were also observed in the mesenteric microcirculation *in vivo*, where IRL-1620 induces vasodilatation in control rats, vasoconstriction in male DOCA-salt rats, and minimal changes in vessel diameter in female hypertensive rats (6). In the current study we investigated whether the increased ET-1/ET_B receptor-mediated vascular responses observed in male, but not in female, DOCA-salt rats are associated with gender differences in the vascular mRNA expression of ET-1 and ET_A/ET_B receptors and/or with functional differences in Ca^{2+} handling mechanisms by vascular myocytes. The rationale was based on the observations that i) changes in both receptor function or intracellular Ca^{2+} regulation are implicated in altered vascular reactivity in hypertension; ii) a defect in intracellular Ca^{2+} regulation, characterized by increased basal tone, increased L-type Ca^{2+} channel activity and altered mobilization of Ca^{2+} from intracellular stores, has been extensively described in the vasculature of male DOCA-salt hypertensive rats (11,12,15); iii) ET-1 has been shown to exert a wide range of effects on some of these defective mechanisms, activating Ca^{2+} influx mainly through VOC and also stimulating IP_3-dependent and (to a lesser extent) IP_3-independent Ca^{2+} release from intracellular stores (3-5);

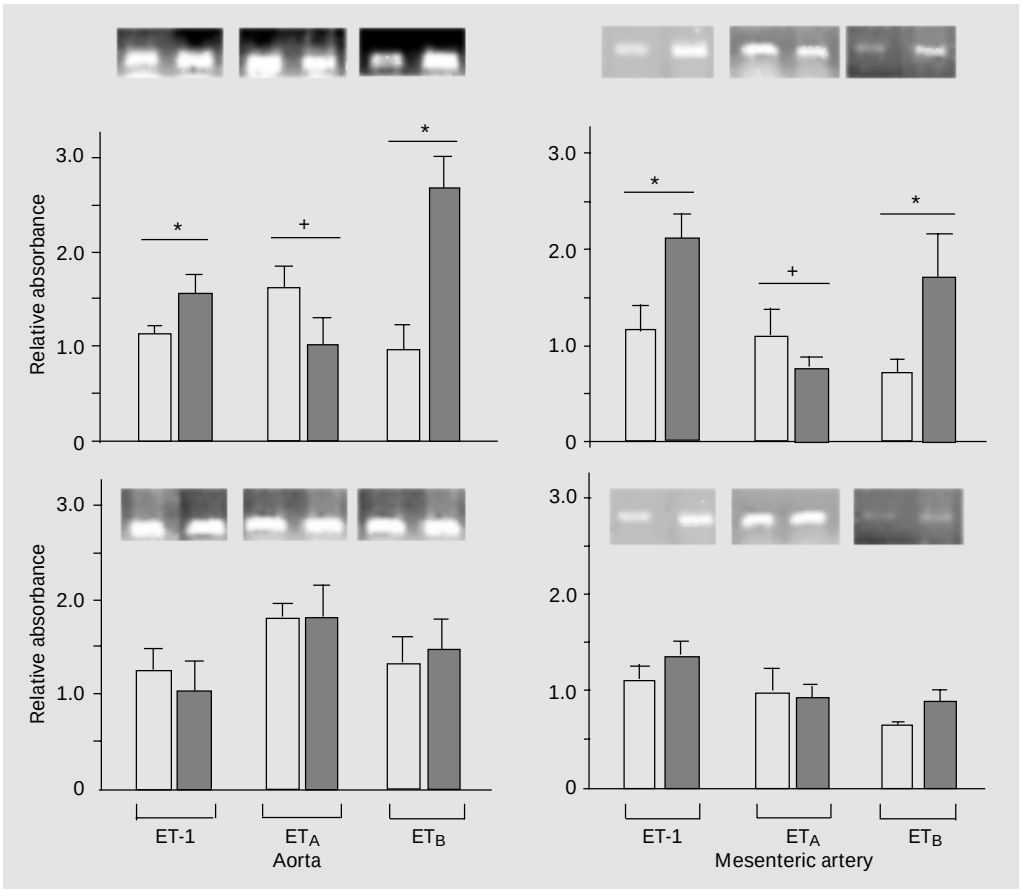

Figure 3. Representative RT-PCR products of 20 ng total RNA extracted from aortas and mesenteric arteries of male (top) and female (bottom) control (open columns) and DOCA-salt (filled columns) rats. The bar graphs show the relative absorbance values of ET-1, ET_A and ET_B receptor bands obtained for the different groups. Values were normalized by the corresponding GAPDH amplicons, used as internal standard. Results are reported as means ± SEM and are representative of 4-5 experiments. *P<0.05 *vs* respective control; $^+$indicates borderline significance (P = 0.052 and P = 0.06 in aortas and mesenteric arteries, respectively) *vs* respective control (*t*-test).

iv) changes in the ratio of ET_A/ET_B receptors have also been described in tissues from male DOCA-salt rats (9,10).

Our results show that arteries from female DOCA-salt rats display changes in Ca^{2+} handling mechanisms similar to those observed in arteries from male DOCA-salt rats: i) increased contractile responses to Bay K8644, ii) greater contractions to caffeine, iii) contractile activity during the Ca^{2+} loading period, and iv) increased caffeine- or agonist-induced contractions in Ca^{2+}-free buffer. These results suggest, therefore, that the increased vascular sensitivity to ET-1/IRL-1620 observed in male DOCA-salt hypertensive rats is not related to gender differences in Ca^{2+} handling mechanisms by vascular myocytes or in the mechanisms activated by ET-1 to increase $[Ca^{2+}]_i$.

An explanation for the increased sensitivity of arteries from male DOCA-salt rats to ET-1/IRL-1620 was an increased expression of vascular ET_B receptors. To evaluate this possibility, we performed RT-PCR in vessels from male and female control and DOCA-salt rats. We found that aortas and mesenteric arteries from male, but not female, DOCA-salt rats display increased ET-1 mRNA expression in comparison to arteries from control rats. Possibly due to a counter-regulatory mechanism, ET_A receptor gene expression was slightly decreased (to a borderline significant level) and gene expression of the ET_B receptor subtype was augmented in vessels from male hypertensive rats. These changes were not observed in vessels from female DOCA-salt rats, confirming our previous data showing that only arteries from male DOCA-salt rats exhibit marked vasoconstriction in response to the selective ET_B receptor agonist IRL-1620 (6). Increased ET-1 content evaluated by ET-1 mRNA levels and immunoreactive ET-1 in blood vessels from male DOCA-salt rats has been previously reported (7,8). Changes in the expression or ratio of ET-1 receptors, exemplified by decreased binding of ET-1 in mesenteric arteries (16), decreased density of cardiomyocyte ET_A receptors and fibroblast ET_B receptors (9), as well as increased expression of ET_B receptors in the renal medulla (10), have also been reported in DOCA-salt hypertension. To our knowledge, however, this is the first report that describes increased gene expression of ET_B receptors in vessels from DOCA-salt rats.

The attenuated changes of ET-1 and ET_A/ET_B expression in arteries from female DOCA-salt rats may be related to the modulation exerted by the gonadal hormones on the ET-1 system. Gender differences in endothelin receptor density and in the relative proportion of receptor subtypes in humans (17), as well as increased expression of prepro-ET-1 mRNA in porcine aortic endothelial cells in the absence of female ovarian hormones (18), have been reported. In addition, it has been shown that 17ß-estradiol attenuates ET-1-induced coronary artery constriction both *in vitro* (19) and *in vivo* (20), and influences the affinity of endothelin receptors in coronary arterial smooth muscle (21).

This study demonstrated that changes in vascular expression of mRNA for ET-1 and ET_A/ET_B receptors occur in male, but not in female, DOCA-salt rats, whereas the Ca^{2+} handling mechanisms by vascular myocytes are similarly altered in arteries from male and female DOCA-salt rats. The molecular up-regulation of vascular ET_B receptors seems to account for the increased vascular reactivity to ET-1 and ET_B receptor-selective agonists observed in male DOCA-salt rats and may well play a role in the higher blood pressure levels observed in male DOCA-salt hypertensive rats.

Acknowledgments

We thank Sonia M.R. Leite for excellent technical assistance.

References

1. Kanaide H (1996). Endothelin regulation of vascular tonus. *General Pharmacology*, 27: 559-563.
2. Schiffrin EL & Touyz RM (1998). Vascular biology of endothelin. *Journal of Cardiovascular Pharmacology*, 32 (Suppl 3): S2-S13.
3. Douglas AS & Ohlstein EH (1997). Signal transduction mechanisms mediating the vascular actions of endothelin. *Journal of Vascular Research*, 34: 152-164.
4. Griendling KK, Tsuda T & Alexander RW (1989). Endothelin stimulates diacylglycerol accumulation and activates protein kinase C in cultured vascular smooth muscle cells. *Journal of Biological Chemistry*, 264: 8237-8240.
5. Fluckiger J-P, Nguyen PV, Li G, Yang X-P & Schiffrin EL (1992). Calcium phosphoinositide, and 1,2 diacylglycerol responses of blood vessels of deoxycorticosterone-acetate hypertensive rats to endothelin-1. *Hypertension*, 19: 743-748.
6. Tostes RCA, David FL, Fortes ZB, Nigro D, Scivoletto R & Carvalho MHC (2000). Deoxycorticosterone acetate-salt hypertensive rats display gender-related differences in ET_B receptor-mediated vascular responses. *British Journal of Pharmacology*, 130: 1092-1098.
7. Larivière R, Day R & Schiffrin EL (1993). Increased expression of endothelin-1 gene in blood vessels of deoxycorticosterone acetate-salt hypertensive rats. *Hypertension*, 21: 916-920.
8. Larivière R, Thibault G & Schiffrin EL (1993). Increased endothelin-1 content in blood vessels of deoxycorticosterone acetate-salt hypertensive but not in spontaneously hypertensive rats. *Hypertension*, 21: 294-300.
9. Fareh J, Touyz RM, Schiffrin EL & Thibault G (2000). Altered cardiac endothelin receptors and protein kinase C in deoxycorticosterone-salt hypertensive rats. *Journal of Molecular and Cellular Cardiology*, 32: 665-676.
10. Pollock DM, Allcock GH, Krishnan A, Dayton BD & Pollock JS (2000). Upregulation of endothelin B receptors in kidneys of DOCA-salt hypertensive rats. *American Journal of Physiology*, 278: F279-F286.
11. Watts SW, Finta KM, Lloyd MC, Storm DS & Webb RC (1994). Enhanced vascular responsiveness to Bay K8644 in mineralocorticoid- and N-nitro arginine-induced hypertension. *Blood Pressure*, 3: 340-348.
12. Tostes RCA, Traub O, Bendhack LM & Webb RC (1995). Sarcoplasmic reticulum Ca^{2+} uptake is not decreased in aorta from DOCA hypertensive rats: Functional assessment with cyclopiazonic acid. *Canadian Journal of Physiology and Pharmacology*, 73: 1536-1545.
13. Leitjen PAA & van Breemen C (1984). The effects of caffeine on the noradrenaline-sensitive Ca^{2+} store in rabbit aorta. *Journal of Physiology*, 357: 327-339.
14. Yamamoto H, Hwang O & Van Breemen C (1984). Bay K8644 differentiates between potential and receptor operated Ca^{2+} channels. *European Journal of Pharmacology*, 102: 555-557.
15. Tostes RC, Wilde DW, Bendhack LM & Webb RC (1997). Calcium handling by vascular myocytes in hypertension. *Brazilian Journal of Medical and Biological Research*, 30: 315-323.
16. Nguyen PV, Parent A, Deng LY, Fluckiger JP, Thibault G & Schiffrin EL (1992). Endothelin vascular receptors and responses in deoxycorticosterone acetate-salt hypertensive rats. *Hypertension*, 19 (Suppl II): II-98-II-104.
17. Ergul A, Shoemaker K, Puett D & Tackett RL (1998). Gender differences in the expression of endothelin receptors in human saphenous vein *in vitro. Journal of Pharmacology and Experimental Therapeutics*, 285: 511-517.
18. Wang X, Barber DA, Lewis DA, McGregor CGA, Sieck GC, Fitzpatrick LA & Miller VM (1997). Gender and transcriptional regulation of NO synthase and ET-1 in porcine aortic endothelial cells. *American Journal of Physiology*, 273: H1962-H1967.
19. Lamping KG & Nuno DW (1996). Effects of 17ß-estradiol on coronary microvascular responses to endothelin-1. *American Journal of Physiology*, 271: H1117-H1124.
20. Sudhir K, Ko E, Zellner C, Wong HE, Hutchison SJ, Chou TM & Chatterjee K (1997). Physiological concentrations of estradiol attenuate endothelin-1-induced coronary vasoconstriction *in vivo*. *Circulation*, 96: 3626-3632.
21. Barber DA, Michener SR, Ziesmer SC & Miller VM (1996). Chronic increases in blood flow upregulate endothelin-B receptors in arterial smooth muscle. *American Journal of Physiology*, 270: H65-H71.