Published in final edited form as:

J Am Soc Hypertens. 2008 November ; 2(6): 439–447. doi:10.1016/j.jash.2008.07.001.

Murine and rat cavernosal responses to endothelin-1 and urotensin-II Vasoactive Peptide Symposium

Fernando S. Carneiro^{1,2}, Zidonia N Carneiro¹, Fernanda R.C. Giachini^{1,2}, Victor V Lima¹, Edson Nogueira¹, William E Rainey¹, Rita C. Tostes^{1,2}, and R. Clinton Webb¹

¹ Medical College of Georgia, Department of Physiology, Augusta, GA, USA

² Department of Pharmacology, Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo, SP, Brazil

Abstract

Background—Endothelin-1 (ET-1) and urotensin-II (U-II) are the most potent constrictors of human vessels. Although the cavernosal tissue is highly responsive to ET-1, no information exists on the effects of U-II on cavernosal function. The aim of this study was to characterize ET-1 and U-II responses in corpora cavernosa from rats and mice.

Methods and Results—Male Wistar rats and C57/BL6 mice were used at 13 weeks. Cumulative concentration-response curves to ET-1, U-II and IRL-1620, an ET_B agonist, were performed. ET-1 increased force generation in cavernosal strips from mice and rats, but no response to U-II was observed in the presence or absence of L-NAME, or in strips pre-stimulated with 20mM KCl. IRL-1620 did not induce cavernosal contraction even in presence of L-NAME, but induced a cavernosal relaxation which was greater in rats than mice. No relaxation responses to U-II were observed in cavernosal strips pre-contracted with phenylephrine. mRNA expression of ET-1, ET_A, ET_B and U-II receptors, but not U-II was observed in cavernosal strips.

Conclusion—ET-1, via ET_A receptors activation, causes contractile responses in cavernosal strips from rats and mice whereas ET_B receptor activation produces relaxation. Although the cavernosal tissue expresses U-II receptors, U-II does not induce contractile responses in corpora cavernosa from mice or rats.

Keywords

endothelin-1; ET_A receptor; urotensin-II; DOCA-salt hypertension; erectile dysfunction; corpus cavernosum

INTRODUCTION

The balance between contracting and relaxing factors controls smooth muscle tone of both the penile vasculature and corpora cavernosa and, therefore, determines the functional state of the penis: flaccidity or erection [1–3]. Neurogenic nitric oxide (NO) is considered the most important factor for relaxation of penile vessels and corpus cavernosum and, consequently,

Corresponding author: Fernando S Carneiro, Medical College of Georgia, Department of Physiology, 1120 Fifteenth Street, CA-3141, Augusta, GA 30912-3000, Phone: +1 (706) 721-0784 Fax: +1 (706) 721-7299, fcarneiro@mcg.edu.

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penile erection. On the other hand, it is generally accepted that the penis is kept in the flaccid state mainly via a tonic activity of noradrenaline derived from the sympathetic nervous system and endothelins, whereas other transmitters or vasoactive molecules, such as neuropeptide Y, angiotensin II and contractile prostanoids, would play a minor role [1–3].

Granchi and colleagues showed that penile smooth muscle cells not only respond to, but also synthesize, endothelin-1 (ET-1) [4]. The authors reported that both endothelial and smooth muscle cells of the human adult and fetal penis express ET-1, its converting enzyme (ECE-1) and predominantly express the ET_A receptor subtype, with ET_B receptors being less expressed [4]. In addition, ET-1 expression in human fetal penile smooth muscle cells is increased by several stimuli, such as transforming growth factor- β 1 (TGF- β 1), interleukin-1 α (IL-1 α), ET-1 itself and prolonged hypoxia [4].

ET-1 receptors, both ET_A and ET_B, have been reported in the cavernosal tissue of humans [4–6] and different animal species (rabbit [6–8], rat [9,10] and bovine [11]). In the rat, as an example, ET-1 injection into the penile vasculature induces both vasoconstriction and vasodilation [12]. ET-1-induced cavernous vasoconstriction *in vivo* seems to be predominantly mediated by the ET_A receptor, since ET_A, but not ET_B, receptor antagonists, prevent ET-1-induced decrease of intracavernosal pressure, both in basal conditions or upon submaximal ganglionic stimulation [10]. In the penis, ET-1/ET_A receptor-mediated biological effects involve activation of the inositol trisphosphate (IP₃)/calcium (Ca²⁺) and RhoA/Rho-kinase signaling pathways [13,14], whereas ET-1/ET_B receptor-mediated vasodilation occurs via release of NO from cavernous endothelial cells [12].

ET-1 not only induces vasoconstriction, but it also stimulates the production of growth factors as well as deposition of extracellular matrix components; it induces generation of reactive oxygen species (ROS); stimulates the expression of adhesion molecules by endothelial cells, potentiates monocyte migration, and activates transcriptional factors responsible for the coordinated increase in the expression of many cytokines and enzymes, leading to the production of inflammatory and tissular damage mediators [15,16].

Urotensin-II (U-II) has been identified as an endogenous ligand for the orphan G-protein coupled receptor 14 (GPR-14), initially named sensory epithelium neuropeptide-like receptor [17,18]. Stimulation of U-II receptors in vascular smooth muscle cells leads to activation of Gq protein, PKC and PLC enzymes, with the subsequent production of the second messengers IP₃ and DAG [19]. Production of these messengers leads to release of Ca²⁺ from the sarcoplasmic reticulum, extracellular Ca²⁺ influx, activation of Ca²⁺-calmodulin-dependent myosin light chain kinase (MLCK), and vasoconstriction. The vasoconstrictor effects of U-II are also mediated by ERK1/2 and RhoA/Rho kinase-related pathways [19,20]. In contrast, U-II has been demonstrated to be an endothelium-dependent vasodilator, acting through release of NO, PGI₂, prostaglandin E₂ (PGE₂), and endothelium-derived hyperpolarizing factor (EDHF) [21]. Unlike ET-1, which uniformly constricts most blood vessels, the vasoactive effects of U-II depend both on the species and vascular bed being considered.

Whereas ET-1 actions on cavernosal tissue have been studied, the (patho)physiological role of U-II in cavernosal tissue contractility remains unclear. Considering that U-II is a potent vasoconstrictor peptide and its vasoconstrictive potency can be greater than that of ET-1 (amounting to eight- to 110-fold the potency of ET-1) [17], we aimed to compare the cavernosal tissue responses to the most potent vasoconstrictor peptides known to date: ET-1 and U-II. Given that U-II displays differential vasoconstrictor effects among several species and vascular beds [22], we determined cavernosal responses to U-II and ET-1 in rats and mice.

In addition, because hypertension is a risk factor for erectile dysfunction (ED) [23], and ET-1 is important in salt-sensitive forms of experimental hypertension, such as mineralocorticoid

hypertension [15,16], it is possible that vascular responses to U-II are altered in a pathological condition such as DOCA-salt hypertension. Therefore, the present study also determined whether DOCA-salt hypertensive animals display abnormal cavernosal reactivity to U-II.

METHODS

Animals

Male Wistar rats (13 weeks-old; Harlan, Indianapolis, IN) and male C57BL/6 mice (13 weeks-old; Harlan, Indianapolis, IN) were used in these studies. All procedures were performed in accordance with the Guiding Principles in the Care and Use of Animals, approved by the Medical College of Georgia Committee on the Use of Animals in Research and Education. The animals were housed four per cage on a 12-h light/dark cycle and fed a standard chow diet with water *ad libitum*.

Functional Studies in Cavernosal Strips

After euthanasia, penes were excised, transferred into ice-cold buffer, and dissected to remove the tunica albuginea, as previously described [24]. One crural strip preparation (1×1×10 mm) was obtained from each corpus cavernosum (two crural strips from each penis). Cavernosal strips were mounted in 4-ml myograph chambers (Danish Myo Technology, Aarhus, Denmark) containing buffer at 37°C and continuously bubbled with a mixture of 95% O₂ and 5% CO₂. The tissues were stretched to a resting force of 3.0 mN and 2.5 mN in rats and mice, respectively, and allowed to equilibrate for 60 min. Changes in isometric force were recorded using a PowerLab/8SP data acquisition system (Chart software, version 5.0; ADInstruments, Colorado Springs, CO). To verify the contractile ability of the preparations, a high potassium chloride (KCl) solution (120 mM) was added to the organ baths at the end of the equilibration period. Cumulative concentration-response curves to ET-1 (10⁻⁹ M to 3×10⁻⁶ M), IRL-1620 (10⁻¹⁰ M to 10⁻⁶ M; selective ET_B agonist), and U-II (10⁻⁹ M to 10⁻⁴ M; U-II receptor agonist) were performed in both in presence or absence of L-NAME (10⁻⁴ M; nitric oxide synthase inhibitor) and indomethacin (3×10⁻⁶ M; cyclooxygenase inhibitor) with cavernosal strips under basal tone. In order to verify whether U-II could produce a contractile response in cavernosal strips under influence of a facilitatory stimuli, cumulative concentration-response curves to U-II (10⁻⁹ M to 10⁻⁴ M) were performed in cavernosal strips pre-incubated with 20 mM KCl. This concentration of KCl was used based on the fact that it does not induce contractile responses in cavernosal strips by itself. Cumulative concentration-response curves to U-II (10⁻⁹ M to 10⁻⁴ M), and IRL-1620 (10⁻¹⁰ M to 10⁻⁶ M; selective ET_B agonist) were also performed in cavernosal strips pre-contracted with phenylephrine (PE; 10⁻⁵ M). When agonists or inhibitors were used, drugs were introduced 30–45 minutes before concentration-response curves were performed. Time control experiments were performed to determine force development of cavernosal strips not related to the effects of each agonist. Control solutions containing vehicle levels of ethanol and DMSO were also used through the experimental protocols.

RNA extraction, cDNA synthesis, and quantitative Real-Time Reverse-Transcriptase Polymerase Chain Reaction

Total RNA was extracted from cavernosal strips using the RNeasy Kit (Qiagen Sciences, Maryland, USA). The quantity, purity and integrity of all RNA samples were determined by the NanoDrop spectrophotometers (NanoDrop Technologies, Wilmington, DE). One microgram of total RNA was reverse transcribed in a final volume of 50 µL using the High-Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA). The single-strand cDNA was stored at -20°C. Primers for preproendothelin-1 (preproET-1 for mouse: cat# Mn00438656_m1), (preproET-1 for rat: cat# Rn00561129_m1), ET_A (ET_A for rat: cat# Rn00561137_m1), (ET_B for rat: cat# Rn00569139_m1), U-II (U-II for rat: cat# Rn00569745_m1) and U-II (U-II for rat: Rn00571932_s1) mRNA receptors were obtained

from Applied Biosystems. Real-time reverse-transcriptase polymerase chain reaction (qPCR) reactions were performed using the 7500 Fast Real-Time PCR System (Applied Biosystems) in a total volume of 20 μ L reaction mixture following the manufacturer's protocol, using the TaqMan[®] Fast Universal PCR Master Mix (2X) (Applied Biosystems), and 0.1 μ M of each primer. Negative controls contained water instead of first-strand cDNA. Each sample was normalized on the basis of its 18S ribosomal RNA content. The 18S quantification was performed using a TaqMan Ribosomal RNA Reagent Kit (Applied Biosystems) following the manufacturer's protocol. Results were calculated using the $\Delta\Delta C_t$ method and expressed as *n*-fold differences in preproET-1 gene expression relative to 18S rRNA and to the calibrator and were determined as follows: $n\text{-fold} = 2^{-(\Delta C_t \text{ sample} - \Delta C_t \text{ calibrator})}$, where the parameter C_t (threshold cycle) is defined as the fractional cycle number at which the PCR reaction reporter signal passes a fixed threshold. ΔC_t values of the sample and the calibrator were determined by subtracting the average C_t value of the transcript under investigation from the average C_t value of the 18S rRNA gene for each sample.

DOCA-salt hypertension and arterial blood pressure measurements

DOCA-salt hypertension was induced as previously described [25]. Briefly, rats and mice were uninephrectomized and deoxycorticosterone-acetate (DOCA rats; 200mg/Kg or DOCA mice; 1g/Kg) silastic pellets were implanted subcutaneously at the scapular region. DOCA animals received water containing 1% NaCl and 0.2% KCl, for 5 weeks. Control animals were also uninephrectomized, received silastic pellets without DOCA and normal tap water. Systolic blood pressure (SBP) was measured in non anaesthetized animals by tail cuff using a RTBP1001 blood pressure system (Kent Scientific Corporation, Connecticut). At the end of 5 weeks of treatment, rats and mice cavernosal strips were submitted to the experimental procedures.

Drugs and Solutions—Physiological salt solution of the following composition was used: 130 mM NaCl, 14.9 mM NaHCO₃, 5.5 mM dextrose, 4.7 mM KCl, 1.18 mM KH₂PO₄, 1.17 mM MgSO₄·7H₂O, 1.6 mM CaCl₂·2H₂O, and 0.026 mM EDTA. N ω -nitro-L-arginine methyl ester (L-NAME), phenylephrine, indomethacin and urotensin-II were purchased from Sigma Chemical Co. (St. Louis, MO). Endothelin-1 and IRL-1620 [Suc-[Glu⁹,Ala^{11,15}]-endothelin-1 (8–21)] were from Tocris (Ellisville, MO). All reagents used were of analytical grade. Stock solutions were prepared in deionized water, and stored in aliquots at -20°C ; dilutions were made up immediately before use.

Statistical Analysis—Contractions were recorded as changes in the displacement from baseline and are represented as millinewtons (mN) for *n* experiments. Relaxation is expressed as percentage change from the PE-contracted levels. Agonist concentration–response curves were fitted using a nonlinear interactive fitting program (Graph Pad Prism 4.0; GraphPad Software Inc., San Diego, CA, USA). Agonist potencies and maximum responses are expressed as pD₂ (negative logarithm of the molar concentration of agonist producing 50% of the maximum response) and E_{max} (maximum effect elicited by the agonist), respectively. Statistically significant differences were calculated by one-way analysis of variance (ANOVA) or Student's *t*-test. $P < 0.05$ was considered as statistically significant.

RESULTS

The weight of the male Wistar rats and C57BL/6 mice (at 13 weeks) was 390 ± 14 ($n=13$) and 27.2 ± 0.7 grams ($n=15$), respectively. Stimulation with KCl 120 mM induced a contractile response of 1.76 ± 0.36 and 0.83 ± 0.12 mN in cavernosal strips from rats and mice, respectively.

Corpora cavernosa responses to ET-1 and U-II

In the first set of experiments, we evaluated responses induced by ET-1 and U-II in cavernosal tissue. Cumulative addition of ET-1 to the bathing caused contraction of rats and mice cavernosal segments, which consisted of a rapid rise in force followed by a slower rise to a sustained level within 10 min. Contractile responses to ET-1, which are nearly abolished by the addition of atrasentan (10^{-6} M), an ET_A antagonist, were observed in all strips from rats and mice (Emax: 0.97 ± 0.07 and 0.79 ± 0.09 mN, respectively; Figure 1). Cumulative addition or a single dose (10^{-4} M) of U-II did not induce contractile responses in cavernosal strips from rats (Figure 1A) or mice (Figure 1B). U-II did not produce cavernosal contractile responses even after incubation of the preparations with L-NAME (10^{-4} M) plus indomethacin (3×10^{-6} M) or 20 mM KCl (data not shown).

Considering that activation of smooth muscle cell ET_B receptors induces contraction whereas endothelial cell ET_B receptors stimulates the release of NO and prostacyclin, and, consequently, induces vasodilation, responses to the ET_B agonist IRL-1620 were tested both under basal tone and after cavernosal strips were stimulated with PE (10^{-5} M). IRL-1620 did not induce contractile responses when tested in tissues at basal tone, either in the absence or presence of L-NAME (10^{-4} M) (Figure 2A), but produced relaxation of cavernosal strips contracted with PE (10^{-5} M) (Figure 2B). IRL-1620-induced relaxation was greater in corpora cavernosa from rats compared to mice (pD_2 : 8.72 ± 0.18 and 7.82 ± 0.5) (Table 1). U-II not only binds to receptors in smooth muscle cells, causing contraction, but also induces endothelium-dependent vasodilation, via release of NO, PGI_2 , prostaglandin E_2 (PGE_2), and endothelium-derived hyperpolarizing factor (EDHF) [21]. In order to verify whether U-II produces relaxation in cavernosal tissue, cumulative concentration-response curves to U-II were performed in cavernosal strips pre-contracted with PE (10^{-5} M). The cumulative addition of U-II was not able to produce either relaxation or additional contraction in pre-contracted cavernosal strips from rats or mice (Figure 2C). Although U-II can induce fairly rapid (minutes) contraction of isolated blood vessels, it may take up to 2 hours for changes in regional vascular resistance to become apparent in the rat.[26] Considering this, experiments were carried out to determine if U-II causes contractile response of corpora cavernosa after 2 hours of incubation. However, U-II did not evoke either contractile response or spontaneous tone development in cavernosal tissue from rats (data not shown).

ET-1, ET_A and ET_B receptors, U-II and U-II receptor mRNA expression in corpora cavernosa

Considering that the lack of functional responses to U-II in cavernosal tissue could be due to the absence of U-II receptors, in the second set of experiments we evaluated whether cavernosal strips from rats display gene expression of U-II and ET-1 and their respective receptors. Consistently with the functional data, cavernosal strips from rats display ET-1, ET_A and ET_B mRNA expression at similar levels of aortic tissue (Figure 3A,B and C, respectively). Although there was a tendency of greater ET-1 expression in cavernosal tissue than in the aorta, it was not statistically significant ($p=0.0688$; Figure 3A). For U-II expression adrenal glands were used as a positive control. U-II expression was not detected in cavernosal tissue (Figure 3D), kidney or aorta (data not shown) from rats. Despite this fact, U-II receptors were expressed in the cavernosal tissue, with levels fivefold greater than in aorta (Figure 3E). In addition, DOCA-salt treatment did not change U-II receptors expression in corpora cavernosa (Figure 3F).

Effects of DOCA-salt hypertension on cavernosal responses to U-II

In the third set of experiments we tested the hypothesis that U-II plays a role in cavernosal reactivity under a pathological condition such as DOCA-salt hypertension. After 5 weeks of treatment, systolic blood pressure (SBP), measured indirectly by plethysmography tail-cuff in conscious animals, was higher in DOCA-salt animals in comparison to their respective controls

(Table 2). Stimulation with 120 mM KCl induced a contractile response (mN) of 1.81 ± 0.33 (n=5) and 2.26 ± 0.49 (n=5) in strips from control and DOCA-salt rats, respectively. Contractile responses (mN) of 0.81 ± 0.11 (n=10) and 0.86 ± 0.1 (n=10) to 120 mM KCl were obtained in strips from control and DOCA-salt mice, respectively.

Although contractile responses to ET-1 were again observed in strips from DOCA-salt rats and mice (Figure 4A) and, DOCA-salt treatment enhanced contractile response to ET-1 compared to their respective controls (Table 1), no contractile responses were observed in cavernosal strips from DOCA-salt rats or mice to U-II (Figure 4B). Cumulative concentration-response curves to U-II were performed both in the presence or absence of L-NAME (10^{-4} M) plus indomethacin (3×10^{-6} M) or after a facilitatory stimulus of 20 mM of KCl in cavernosal strips from DOCA-salt animals. However, U-II was not able to elicit contractile response in corpora cavernosa under these conditions (data not shown). To evaluate U-II integrity, concentration-response curves were performed in rat aorta, which is known to contract to U-II. Aorta of rats did exhibit contractile response to U-II, confirming that the drug was active (Figure 4C).

DISCUSSION

The findings of this study demonstrate that whereas corpora cavernosa from rats and mice display contractile responses to ET-1 via activation of ET_A receptors, ET_B receptor activation produces cavernosal relaxation. In addition, we demonstrated that U-II does not induce contractile or relaxant responses in cavernosal strips from these animals, neither in physiological or in a pathological condition such as DOCA-salt hypertension.

ET-1 and U-II are the most potent and unusually long-lasting constrictors of human vessels known to date [27]. ET-1, which potently induces slowly developing, long-lasting contractions in the corporal smooth muscle cells and penile vessels, has been suggested to contribute to the maintenance of corpus cavernosum smooth muscle tone [1–3,12–14]. On the other hand, the role of urotensin II in cardiovascular (patho)physiology is not well established.

Considering that the balance between contracting and relaxing factors controls smooth muscle tone of both the penile vasculature and corpora cavernosa and, therefore, determines the functional state of the penis: flaccidity or erection [1–3], it is rational to ask whether U-II plays a role in the control of cavernosal smooth muscle tone. Based on that, the present study aimed to compare the responses of ET-1 and U-II in cavernosal tissue. In addition, considering that the vasoactive effects of U-II depend both on the species and vascular bed being considered, we performed our study in two different animal species: rats and mice.

In the first set of experiments, we observed that cavernosal strips from rats and mice displayed contractile responses to ET-1. In accordance to our results, other reports have demonstrated that ET-1 potently induces long-lasting contractions in penile smooth muscle tissue [28], cavernous artery, deep dorsal vein [29] and penile circumflex veins [6]. Contractions were also evoked in human corpus cavernosum by ET-2 and ET-3, although these peptides had a lower potency than ET-1 [5]. In the present study we observed that ET-1-induced contraction was completely abolished by atrasentan (ET_A receptor antagonist). These results suggest that ET-1-induced contraction is mainly mediated via ET_A receptors in cavernosal tissue. In addition, our results showing that IRL-1620 (ET_B receptors agonist) induces relaxation, but not contraction, of cavernosal strips suggest the presence of endothelial cell ET_B receptors and are in accordance with a previous report showing that, in the rat, ET-1 injection into the penile vasculature induces both vasoconstriction and vasodilation [12]. Although the ET_A receptors seem to mediate ET-1-induced contractions, in cavernosal tissue from rabbits ET_A receptor antagonist BQ-123, did not appear to affect the resting tension [29]. A pilot study with an ET_A receptor antagonist, BMS-193884, for the treatment of mild to-moderate ED, showed that in anesthetized male

rabbits, intravenous administration of BMS-193884 increases the duration of pelvic nerve-stimulated penile erection [30]. However, in men diagnosed with mild-to-moderate ED, administration of BMS-193884 did not significantly improve erectile function [30]. It is possible that ET-1 is involved in ED associated with specific conditions (diabetes- or salt-sensitive hypertension-associated ED) and that ET_A or dual ET_A/ET_B antagonists may be beneficial only in these subset of patients.

Several pharmacological approaches were carried out in order to determine if in different conditions U-II could elicit any relaxant or contractile effect on cavernosal tissue. First, considering that U-II has been demonstrated to be an endothelium-dependent vasodilator, acting through release of NO, PGI₂, prostaglandin E₂ (PGE₂), and endothelium-derived hyperpolarizing factor (EDHF) [21], cumulative concentration-curves to U-II were performed in cavernosal strips from rats and mice pre-incubated with L-NAME plus indomethacin, this protocol would unmask any relaxant response of U-II that could be decreasing contractile responses to the peptide. However, no contractile response was observed. Subsequently, we tested if in the presence of a facilitatory stimulus, 20 mM KCl, U-II would be able to produce contraction in cavernosal tissue, but no response was evoked.

Considering that ET-1 is important in salt-sensitive forms of experimental hypertension, such as mineralocorticoid hypertension [15,16] and salt-sensitive hypertensive animals display increased tissue ET-1 expression and abnormal vascular responses to ET-1, we tested whether responses to U-II are present in a pathological condition, such as DOCA-salt hypertension. It is possible that the increased cavernosal ET-1 content in DOCA-salt hypertension would increase or modulate cavernosal responses to U-II, as is the case for ET-1 modulation effects in human corpus cavernosum [31]. In rats, DOCA-salt treatment for 5 weeks severely increases blood pressure and decreases erectile function [25]. Although receiving a dose of deoxycorticosterone 5 times greater (1 g/Kg) than that normally used to treat rats (200 mg/Kg), DOCA-salt mice displayed only a moderate increase in blood pressure, in accordance with data from other groups [32,33]. Regardless of the effect on level of blood pressure in both species, no contractile or relaxant responses to U-II were obtained in corpora cavernosa. As expected and already demonstrated in vascular tissues, responses to ET-1 were altered in cavernosal strips from DOCA-salt hypertensive animals.

Urotensin-II has been identified as an endogenous ligand for the orphan G-protein coupled receptor 14 (GPR-14), initially named sensory epithelium neuropeptide-like receptor [17,18]. The GPR-14 receptor has seven transmembrane domains and has been identified in a number of mammalian species [34]. Although there is functional evidence on the existence of more than one receptor type for U-II [19], only one UT receptor has been identified by molecular approaches. In addition, the lack of responses to U-II in cavernosal tissue suggests the absence of receptors that could trigger functional activation of the tissue to this peptide. However, we observed that U-II receptor mRNA expression is 5 fold greater in the cavernosal tissue than in the aortic tissue, which displays contractile responses to U-II. However, several post-translational changes in U-II receptor mRNA may account for this apparent discrepancy and additional studies should investigate whether corpora cavernosa express U-II receptor protein. Considering that U-II acts as a mitogenic and/or hypertrophic agent in the cardiovascular system via activation of ERK1/2 and RhoA–Rho kinase pathways [20,35,36], it should be noted that U-II might play a role in the corpus cavernosum even if the peptide does not cause contraction or relaxation.

In summary, our study shows that ET-1 causes contractile responses on cavernosal strips from rats and mice via activation of ET_A receptors. ET_B receptor activation produces cavernosal relaxation. Although U-II receptor mRNA expression is detected in the cavernosal strips from

rats, U-II does not induce contraction or relaxation in corpora cavernosa from mice or rats in both physiological and pathological condition such as DOCA-salt hypertension.

Acknowledgments

This study was supported by grants from the National Institutes of Health (HL71138 and HL74167) and Fundacao de Amparo a Pesquisa do Estado de Sao Paulo – FAPESP, Brazil.

Abbreviations

DOCA-salt	deoxycorticosterone and salt-induced hypertension
EC₅₀	the molar concentration of agonist producing 50% of the maximum response
ED	erectile dysfunction
E_{max}	maximum effect elicited by the agonist
ET-1	endothelin-1
ET_A	endothelin receptor A subtype
ET_B	endothelin receptor B subtype
KCl	potassium chloride
L-NAME	N ω -nitro-L-arginine methyl ester
NO	nitric oxide
pD₂	negative logarithm of EC ₅₀
PE	phenylephrine
ROS	reactive oxygen species
U-II	urotensin-II

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Figure 1.

Responses of cavernosal strips from rats and mice to ET-1 and U-II. Representative traces showing a concentration-response curve to ET-1 and U-II in cavernosal strips from mice (A) and rats (B). Cavernosal contractile response curves to ET-1 (C) and U-II (D) in rats (■) and mice (●). Experimental values of ET-1-induced contraction of cavernosal strips are in mN (n=5 in each group). Data represent the mean $\pm$ SEM of n experiments. Arrows indicate the point that drug was added.

Figure 2.

Responses of cavernosal strips from rats and mice to the ET_B agonist IRL-1620 and U-II. (A) Representative traces showing IRL-1620 concentration-response curves at basal tone in the presence of vehicle (upper) or L-NAME 10^{-4} M (bottom). (B) Relaxant responses upon IRL-1620 stimulation in cavernosal strips from rats (■) and mice (●). (C) Representative traces showing concentration-response curves to U-II in cavernosal strips from mice (upper) and rats (bottom). Experimental values of PE-induced contraction of cavernosal strips are in mN ($n=6$ and 7 , respectively) and relaxation induced by IRL-1620 ($n=5$ and 7 , respectively) was calculated relative to the maximal changes from the contraction produced by PE in each tissue, which was taken as 100%. Data represent the mean $\pm$ SEM of n experiments. *, $p<0.05$ rats vs mice. Arrows indicate the point that drug was added.

Figure 3.

Bar graphs showing preproET-1 (A), ET_A (B), ET_B (C), U-II (D) and U-II receptors (E) mRNA expression in cavernosal strips from rats, as determined by qPCR of 1μg total RNA. Negative controls with water instead of first-strand cDNA were used. Aorta was used as a positive control for preproET-1, ET_A, ET_B and U-II receptors. For U-II expression adrenal glands were used as positive control (n=5 to 6 for each gene). In (F), U-II receptors mRNA expression in cavernosal tissue from control and DOCA-salt hypertensive rats. *, p<0.05 aorta vs cavernosum.

Figure 4.

Responses of cavernosal strips from DOCA-salt rats (■) and mice (●) to ET-1 (A) and U-II (B) in DOCA-salt rats (■) and mice (●). Representative trace of rat aorta response to U-II (C). Experimental values of ET-1-induced contraction of cavernosal strips are in mN. Data represent the mean $\pm$ SEM of $n=5$ and 6 , respectively experiments. *, $p<0.05$ compared with values of cavernosal strips from control mice or rats.

Table 1

Emax and pD₂ values for ET-1, IRL-1620 in cavernosal strips from control and DOCA-salt rats and mice.

Drugs	Rats			Mice		
	Control	Emax	pD ₂	DOCA-salt	Control	DOCA-salt
ET-1	6.91±0.120	79±0.09	7.00±0.07	1.25±0.11	*7.1±0.330	46±0.07
IRL-1620	08.72±0.18	68±3	7.64±0.17	*47±3	7.82±0.5	25±3
U-II	n.r	n.r	n.r	n.r	n.r	n.r

Values are means ± SEM for n=5 to 6 in each group. Relaxation induced by IRL-1620 was calculated relative to the maximal changes from the contraction produced by PE, and are represented as % of relaxation. n.r., no-response;

* p<0.05 vs respective control.

Table 2

Systolic blood pressure (SBP) at 5 weeks of treatment, and body weight of control and DOCA-salt hypertensive animals.

Parameters	Rats		Mice	
	Control	DOCA-salt [*]	Control	DOCA-salt [*]
SBP (mmHg)	125 ± 3.6	164 ± 6.4 [*]	103 ± 3	131 ± 4 [*]
Body weight (g)	390 ± 14	319 ± 7 [*]	27.2 ± 0.7	28.3 ± 1.1

SBP, systolic blood pressure; Values are means ± SEM for n= 6 in each group.

*
p<0.05 vs