CLIN. AND EXPER. HYPER.-THEORY AND PRACTICE, A13(5), 807-816 (1991)

INFLUENCE OF SEX ON THE REACTIVITY TO ENDOTHELIN-1 AND NORADRENALINE IN SPONTANEOUSLY HYPERTENSIVE RATS.

Z.B. Fortes; D. Nigro; R. Scivoletto and M.H.C. Carvalho
Department of Pharmacology
Institute of Biomedical Sciences
University of São Paulo, São Paulo, Brazil

Key words: SHR
Endothelin-1
Noradrenaline

ABSTRACT

The response to endothelin-1 and noradrenaline in isolated aortas *in vitro* and mesenteric arterioles *in situ* was studied in male and female spontaneously hypertensive rats (SHR). Greater sensitivity to endothelin-1 and noradrenaline and decreased reactivity to endothelin-1 but not to noradrenaline were found in aortas of male SHR. Mesenteric arterioles of male SHR were more sensitive to endothelin-1 and noradrenaline. Aortas and mesenteric arterioles of female SHR exhibited similar sensitivity to endothelin-1 as compared to controls, whereas a greater reactivity to this agent was only observed in microvessels. It is suggested that sex-linked alterations of vascular reactivity exist in SHR. These alterations seemed not to affect all vascular territories and were not due to differences in blood pressure levels of male and female SHR.

INTRODUCTION

Little is known about the influence of sex steroid hormones in hypertension, although they clearly affect the vascular response to different agents such as catecholamines, angiotensin II, vasopressin etc (1).

A greater sensitivity to endothelin-1 has been demonstrated in isolated renal arteries (2) and in isolated aortas of male SHR (3). Studies on the response of in vitro vessels from SHR to noradrenaline, however, have produced less consistent results. Increased, decreased and no change in the vascular contraction to this agent have been reported (4-7).

There are only a few studies on the vascular response to vasoconstrictor agents in female SHR. A significant increase of aortic reactivity to serotonin of female SHR was found (8). In a previous study we reported similar reactivity to noradrenaline in vessels of female SHR and control rats (9).

Sex-linked alterations of the vascular responses to vasoconstrictor agents, therefore, are not well established in SHR. The present experiments were undertaken to examine the response of macro (aorta) and microvessels (mesenteric arterioles) to endothelin-1 in male and female SHR and to noradrenaline in male SHR.

MATERIAL AND METHODS

Animals

Male and female SHR (14-16 weeks old) and their respective normotensive Wistar controls were used. Tail blood pressures were measured by the tail cuff method in conscious rats. Oestrus was induced in all female rats by injecting subcutaneously stilboestrol tetrasodium acetate (Honvan^{R}) 20 ug/rat in oil solution 24 h before the experiments. Oestrus was determined by microscopic observation of vaginal smears.

Preparation of Isolated Aorta Rings

The rats were anesthetized with chloral hydrate (300 mg.kg i.p.). The thorax was opened and the descending aorta immediately excised.

After removal of loose connective tissue, two transverse rings approximately 4 mm in length were prepared for isometric tension recording in an organ chamber. While one ring served as control, the endothelium was mechanically removed from the other by gently rubbing the luminal surface with a small cylindrical piece of artificial sponge attached to a thread, thus permitting insertion into the lumen. Two L-shaped stainless steel wire hooks were used to mount each ring in the organ bath containing Krebs-Henseleit solution (37°C) saturated with a mixture of 95% O_2 and 5% CO_2. The aorta preparations were allowed to equilibrate for at least 1 hour under a resting tension of 1.5 g, which was maintained throughout the experiment. Developed tension was detected with an F-60 microdisplacement myograph and recorded on a polygraph (Narco Bio-System^R). Cumulative concentration-effect curves were constructed from the response of aortas with and without endothelium to endothelin-1 and noradrenaline dissolved in Krebs-Henseleit solution. When arteries without endothelium were used, effective doses of acetylcholine were added at the end of the experiments to test the efficacy of the procedure for removal of the endothelium. Final molar concentration of the salt in the organ bath are presented. Effective concentrations 50% (EC50) and maximal responses were determined from the cumulative concentration-effect curves.

Preparation of Mesenteric Microvessels In Vivo

The mesentery was exteriorized and arranged for microscopic observation in situ (10). The rats were maintained under chloral hydrate anesthesia (400-450 mg/kg s.c.) at 37°C on a special board containing a transparent plate on which the tissue to be transilluminated was placed. The mesentery was kept moist and warm by irrigating the tissue with Ringer-Locke solution (37°C, pH 7.2-7.4) containing 1% gelatin. In a series of experiments an image splitting micrometer was adjusted to the phototube of the microscope. The image splitter sheared the optical image into two separate images and displaced one with respect to the other. By rotating the image splitter in the phototube the shearing was maintained at right

angles to the axis of the vessel. The displacement of one image from the other allowed measurement of the vessel diameter (11).

First-order arterioles were selected for study and changes in vessel diameter estimated after the topical application of endothelin-1 or noradrenaline (0.01 ml of various molar solutions). In another series of experiments, the preparation was standardized on the basis of the constrictor response to a fixed dose of endothelin-1 or noradrenaline added topically in a volume of 0.01 ml of a molar solution. The response was characterized by the complete cessation of blood flow within 20-50 seconds in at least two vessels (capillaries excluded) of the microscopic field observed at a magnification of x100; the time necessary to impede blood flow (latency) was determined. Synthetic endothelin-1 was obtained from Peptide Institute, Osaka, Japan and noradrenaline bitartrate from Sigma Chemical Co., St Louis, USA.

Statistical Analysis

Data are given as the mean ± SEM. A one-way analysis of variance for repeated measurements and the Student's t test for unpaired data were used where appropriate. The minimum acceptable level of significance was p at a value less than or equal to 0.05.

RESULTS

Blood pressures were elevated significantly in male and female SHR. Tail blood pressures of male rats at the time of the study were 165.5 ± 4.9 (mean ± SEM) and 112.5 ± 3.5 mmHg for 27 SHR and 31 controls respectively. Tail blood pressures of female rats were 166.5 ± 2.8 and 107.1 ± 3.2 mmHg for 22 SHR and 15 controls respectively.

Effect of Endothelin-1 and Noradrenaline in Isolated Aortas

Cumulative concentration-effect curves for endothelin-1 or noradrenaline were obtained simultaneously from the response of two portions of a single artery, one intact and the other lacking endothelium. The effect of endothelin-1 on endothelium-intact

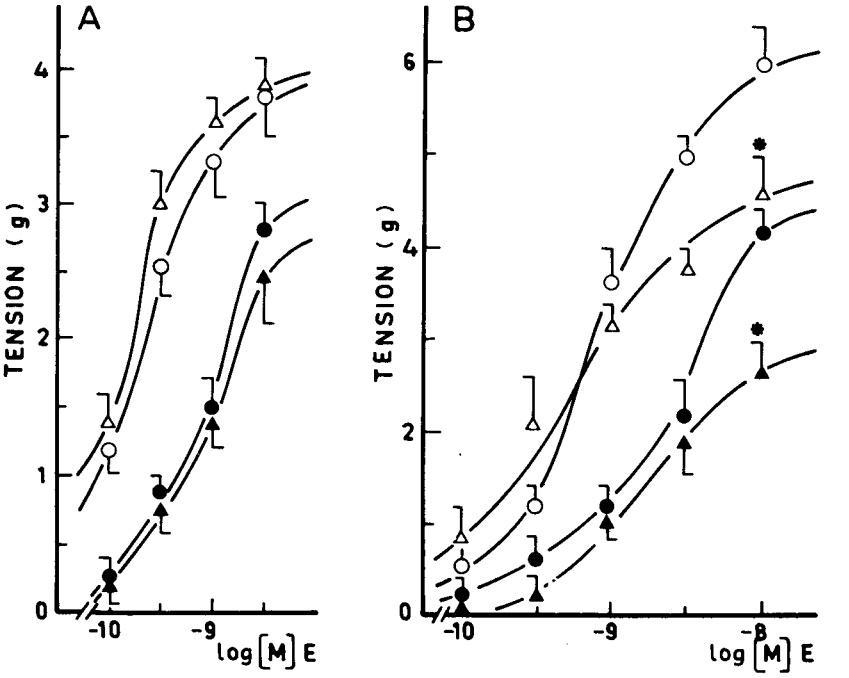

Fig. 1. Cumulative concentration-effect curves to endothelin-1 (E) plotted from the response of two rings of the same aorta: closed symbols, intact endothelium; open symbols, without endothelium. In A (left panel) circles, control female normotensive rats, triangles, female SHR; in B (right panel), circles, control male normotensive rats; triangles, male SHR. Each point represents the mean value for 6 animals in control and SHR groups; abcissae, endothelin-1 concentration expressed in a log scale; ordinate, tension developed by the preparations (g) expressed as mean ± SEM

preparations from female SHR rats was similar to that seen in control rats. Although removal of the endothelium shifted the concentration-effect curves to the left as compared with endothelium-intact preparations, no difference was observed between the response to endothelin-1 in aortas from female SHR and control rats lacking endothelium (fig. 1A).

TABLE 1

Effective concentration 50% (EC50) and maximal response (in g) values for endothelin-1 obtained in aortas with (+) or without (-) endothelium from male SHR and control rats (n=7 in each group).

Group	ENDOTHELIUM (+)		ENDOTHELIUM (-)	
	$EC50x10^{-9}M$	max.resp[a]	$EC50x10^{-9}M$	max.resp.
Control	3.2 (2.6-4.1)	4.5±0.4	0.7[+] (0.4-1.0)	5.9±0.3[+]
SHR	1.0[*] (0.5-1.9)	2.7±0.5[*]	0.3[+] (0.2-0.5)	4.2±0.2[*,+]

a: mean ± SEM

[*] $p < 0.05$ in comparison with respective values in controls.

[+] $p < 0.05$ in comparison with values obtained in respective preparations with endothelium.

In parentheses 95% confidence intervals.

TABLE 2

Effective concentration 50% (EC50) and maximal response (in g) values for noradrenaline obtained in aortas with (+) or without (-) endothelium from male SHR (n=6) or control (n=5).

Group	ENDOTHELIUM (+)		ENDOTHELIUM (-)	
	$EC50x10^{-9}M$	max.resp[a]	$EC50x10^{-9}M$	max.resp.
Control	15.0 (10.0-22.8)	2.8±0.3	0.2[+] (0.1-0.5)	4.1±0.2[+]
SHR	3.8[*] (0.6-24.0)	2.5±0.3	0.6[+] (0.2-1.5)	3.4±0.2[+]

a: values are mean ± SEM

[*] $p < 0.05$ in comparison with values in controls.

[+] $p < 0.05$ in comparison with values obtained in respective preparations with endothelium.

In parentheses 95% confidence intervals.

EC50s and maximal responses to endothelin-1 were significantly lower in male SHR aortas with endothelium in comparison with their respective controls. Removal of the endothelium rendered aortas more sensitive to endothelin-1 than aortas with endothelium in both groups. Smooth muscle reactivity was altered in male SHR since lower maximal response to endothelin-1 was observed in aortas without endothelium (table 1 and fig. 1B). A greater sensitivity to noradrenaline with no alteration of the maximal response was observed in male SHR aortas with endothelium. Smooth muscle reactivity was not altered since aortas without endothelium exhibited similar sensitivity and reactivity (table 2).

Effect of Endothelin-1 and Noradrenaline on Mesenteric Microvessels In Vivo

The initial diameter of the mesenteric arterioles was not different in either male SHR (15.3 ± 0.7 um, n=19) and their respective controls (16.4 ± 1.6 um, n=12) or in female SHR (13.7 ± 0.6 um, n=6) and their respective controls (13.8 ± 0.8 um, n=8).

In control male rat preparations, cessation of blood flow through the mesenteric microvessels was only achieved when endothelin-1 or noradrenaline was applied topically at 10^{-3}M or 3 x 10^{-5}M, respectively (latency 39.7 ± 3.9 sec, n=9 and 17.1 ± 1.9 sec, n=10). In male SHR, 3 x 10^{-9}M endothelin-1 induced cessation of blood flow (latency 44.4 ± 6.8 sec, n=5) and 10^{-5}M noradrenaline (latency 14.7 ± 1.5, n=9). Thus, microvessel preparations of SHR are more sensitive to endothelin-1 and to noradrenaline than are control preparations. This increased sensitivity to endothelin-1 was not observed at 10^{-9}M or at noradrenaline 10^{-6}M where a similar reduction in the initial diameter of arterioles from SHR (18.8 ± 1.3%; n=7 and 14.0 ± 0.5%; n=7) was seen as compared with controls (16.7 ± 1.2%; n=7 and 11.5 ± 2.2%; n=4).

Microvessel preparations from female SHR exhibit sensitivity to endothelin-1 similar to control preparations because complete interruption of blood flow was induced by 10^{-8}M endothelin-1 in both cases. The latency to induce this response, however was

significantly decreased in preparations from SHR as compared with controls (20.8 ± 1.4 sec, n=16 and 38.8 ± 5.7 sec, n=9, respectively). Thus greater reactivity to endothelin-1 was detected in female SHR as compared with control rats. Greater reactivity, however, could not be demostrated at lower concentrations of endothelin-1 (10^{-9}M and 10^{-10}M). Similar decreases in initial diameter (approximately 20% and 10% respectively) were obtained in female SHR and control preparations, at those concentrations.

DISCUSSION

In the present study we demonstrated altered responses to vasoconstrictor agents such as endothelin-1 and noradrenaline in aorta of male but not of female SHR. Altered responses to these agents were also found in mesenteric arterioles of male SHR and to endothelin-1 only in microvessels of female SHR.

The decreased reactivity to endothelin-1 but not to noradrenaline found in aorta of male SHR could indicate that specific alterations might occur that interfere with the response of some but not of all vasoconstrictor agents. In addition, different adjustment mechanisms might be involved that alter differently the responses of small vessels *in situ* and large arteries *in vitro* since decreased reactivity of smooth muscle layer to endothelin-1 was found in aortas but not in arterioles of male SHR.

Sexual hormones might interfere with the vascular responses in hypertension, since vessels from female SHR showed different alterations of the vascular response in comparison with male SHR. Similary to that found to noradrenaline in female SHR (9), aortas and mesenteric arterioles exhibited similar sensitivity to endothelin-1, whereas a greater reactivity to this agent was only observed in microvessels. These differences, however, might not be due to blood pressure levels, since male and female SHR have similar blood pressure levels.

In conclusion, our data allow us to suggest sex-linked alterations of the vascular response to vasoconstrictor agents such as endothelin-1 and noradrenaline in SHR. These alterations did not affect all vascular territories and were not due to differences in blood pressure levels of male and female SHR.

ACKNOWLEDGEMENTS

The expert technical assistance of Hermes Vieira Barbeiro, Maria Aparecida de Oliveira and Sonia Maria Rodriges Leite is fully acknowledge. Research supported in part by FAPESP and FINEP.

REFERENCES

1. ALTURA, B.M. Sex and estrogens and responsiveness of terminal arterioles to neurohypophyseal hormones and catecholamines. J. Pharm. exp. Ther. 1975; **250**:403-412.

2. TOMOBE, Y., MIYAUCHI, T., SAITO, A., YANAGISAWA, M., KIMURA, S., GOTO, K., MASAKI, T. Effect of endothelin on the renal artery from spontaneously hypertensive and Wistar Kyoto rats. Eur. J. Pharm. 1988; **152**:373-374.

3. CLOZEL, M. Endothelin sensitivity and receptor binding in the aorta of spontaneously hypertensive rats. J. Hypertension. 1989; 7:913-917.

4. HAUESLER, G., FINCH, L. Vascular reactivity to 5-hydroxytryptamine and hypertension in the rat. Naunyn-Schmiedeberg's Arch Pharm. 1972; 272:101-106.

5. SPECTOR, S., FLEISCH, J.H., MALING, H.M., BRODIE, B.B. Vascular smooth muscle reactivity in normotensive and hypertensive rats. Science. 1969; **166**:1300-1301.

6. SHIBATA, S., KURAHASHI, K., KUCHH, M. A possible etiology of contractility impairment of vascular smooth muscle from spontaneously hypertensive rats. J. Pharm. exp. Ther. 1973; **185**:406-417.

7. HALLBACK, M., LUNDGREN, Y., WEISS, L. Reactivity of noradrenaline of aortic strips and portal veins from spontaneously hypertensive and normotensive rats. Acta Physiol. Scand. 1971; **81**:176-181.

8. COHEN, M.L., BERKOWITZ, B.A. Decreased vascular relaxation in hypertension. J. Pharm. exp. Ther. 1976; **196**:396-406.

9. CARVALHO, M.H.C., SCIVOLETTO, R., FORTES, Z.B., NIGRO, D., CORDELLINI, S. Reactivity of aorta and mesenteric microvessels to drugs in spontaneously hypertensive rats: role of the endothelium. J. Hypertension. 1987, **5**:377-382.

10. ZWEIFACH, B.W. Indirect methods for regional blood flow I. Microscopic observation of circulation in rat mesoappendix and dog omentum. Use in study of vasotropic substances. Methods Med. Res. 1948; 1:131-138.

11. BAEZ, S. Recording of microvascular dimensions with an image-splitter television microscope. J. Appl. Physiol. 1966; **21**: 299-301.