Ultrastructural characteristics of aortic endothelial cells in borderline hypertensive rats exposed to chronic social stress.

Ľ. OKRUHLICOVÁ¹, K. DLUGOŠOVÁ¹, M. MITAŠÍKOVÁ¹, I. BERNÁTOVÁ²

¹Institute for Heart Research, Slovak Academy of Sciences, 840 05 Bratislava, Slovak Republic, ²Institute of Normal and Pathological Physiology, Center of Excellence for Cardiovascular Research, Slovak Academy of Sciences, 813 71 Bratislava, Slovak Republic

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Corresponding author:
Okruhlicová Ľudmila, PhD.
Institute for Heart Research, Slovak Academy of Sciences
Dúbravská cesta 9, PO Box 104
840 05 Bratislava, Slovak Republic
Tel. + 421 2 54 77 44 04
Fax : + 421 2 54 77 66 37
E-mail: usrdokru@savba.sk
Summary

Genetic predisposition and social stress may represent important risk factors in etiology of hypertension associated with endothelial dysfunction. Perturbations of endothelial structural integrity are also critical to pathogenesis of vascular diseases. We examined effect of chronic social stress on structure of aortic endothelium in borderline hypertensive (BHR) and normotensive Wistar (W) rats. Male BHR - offspring of W mothers and SHR fathers and age-matched W were exposed to 6-week crowding stress (5 rats/cage, 200 cm²/rat). Aortic tissue was processed for electron microscopy and NO synthase activity measurement. Crowding stress significantly increased blood pressure in BHR compared to basal values (140±3 mm Hg vs. 130±3 mm Hg, p<0.05) and reduced enzyme activity by 37% (p<0.01) in the aorta of BHR. Local slight structural alterations of endothelium were found in non-stressed BHR (p<0.001) when compared with W. Chronic stress caused marked (p<0.005) subcellular injury of endothelial cells in aorta of BHR characterized also with mitochondrial damage, presence of vacuoles, increased number of lysosomes, Weibel-Palade bodies, changes of intercellular connections and local disruption of endothelium, while only slight one was seen in W. Results suggest increased sensitivity of aortic endothelium of BHR to chronic crowding that may contribute to acceleration of arterial dysfunction.

Key words.

Social stress, hypertension, endothelium, ultrastructure, rat
Introduction

It is now apparent that endothelial dysfunction referring mainly to impaired NO-dependent vasodilatation precedes clinical symptoms of cardiovascular disease. Besides, endothelial dysfunction is also accompanied with a microscopically visible cell injury as well as disruption of endothelial barrier lining. These structural alterations are implicated as important pathogenic factors contributing to vascular disease states since they affect the permeability of endothelium, allow the passage of circulating blood cells, macromolecules and inflammatory fluid from blood stream to the underlying tissues (Vallet 2003).

Hypertension is a major risk factor of cardiovascular diseases associated with endothelial dysfunction. Structural alterations of endothelium in vessels observed in hypertensive animals (Kristek et al. 1997, Okruhlicova et al. 2000, 2005, Tribulova et al. 2000) indicated their potential contribution to the development or maintenance of the high blood pressure. Hypertension is multifactorial disease, in many cases resulting from complex interaction of genetic and social factors. The use of chronic psychosocial stress in borderline hypertensive rats (BHR) resulted in the development of hypertension, heart hypertrophy and significant cardiac pathology (Lawler et al. 1981). Psychosocial stress is associated with cardiovascular alterations like endothelial dysfunction, inflammation, metabolic and hematological abnormalities, and increased activity of sympathetic nervous system and renin-angiotensin-aldosterone system (McCarty and Gold 1996, Esch et al. 2002). In addition, it was demonstrated from our previous study that altered vascular NO synthesis might also be involved in chronic social stress-induced changes in vascular function and blood pressure in adult borderline hypertensive rats (Bernátová and Csizmadirová 2006).

Though there is some evidence from clinical and experimental studies indicating the impairment of the endothelium-dependent relaxation in hypertension (Törökö et al. 2006) and chronic stress (Sherwood et al. 1999), there is no available information about the fine
structure of endothelium in borderline hypertension exposed to chronic stress. Therefore, we studied the effect of psychosocial stress on endothelial structural integrity of aorta of rats with genetic predisposition to hypertension.

**Material and methods**

*Animals*

BHR used in the study were born in our certified animal facility as F1 offspring of normotensive (Wistar) dams and spontaneously hypertensive sires. All animals BHR and Wistar were housed at 22-24°C on a 12:12-h dark-light cycle (07.00-19.00 lights on) and maintained on a pellet diet and tap water *ad libitum*. After weaning (25th day), male rats were kept in groups of 4 rats per cage (35/55/20 cm, 480 cm²/rat). The investigation conforms to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication NO. 85-23, revised 1996).

*Stress model*

Adult male rats, 15 weeks old, were randomly divided into two groups. Control normotensive and borderline hypertensive rats were kept in groups of 4 rats/cage (35/55/20 cm, 480 cm²/rat). Rats exposed to crowding stress were kept in groups 5 rats/cage (25/49/15 cm), where their living space was reduced to 200 cm²/rat, for 6 weeks (Bernátová and Csizmadiová 2006).

*Blood pressure measurement*

Two weeks before the experiment, the rats were handled and accustomed to the procedure of blood pressure recording, using non-invasive tail-cuff plethysmography. Blood pressure was determined at the beginning and at the end of experiment after 6 weeks.
NO synthase activity

NO synthase activity was determined in crude homogenates of aorta by determination of $[^3H]$-L-citrulline formation from $[^3H]$-L-arginine (Amersham, UK), as described previously (Bernátová et al. 2002).

Transmission electron microscopy

For electron microscopic examination the heart was retrogradely fixed via thoracic aorta with 2.5 % glutaraldehyde in 100 mmol/l cacodylate buffer (pH 7.4). Thoracic aorta was cut into 3 mm long rings and additionally immersely fixed in 2.5 % glutaraldehyde for 3 h at 4°C. After washing in cacodylate buffer, the tissue was postfixed in 1% OsO₄, dehydrated via ethanol series, infiltrated with propylene oxide and embedded in Epon 812. Ultrathin sections cut on ultramicrotome LKB Huxley (London, Great Britain) were counter-stained with uranyl acetate and lead citrate and examined in electron microscope Tesla 500 (Brno, Czech Republic).

Statistical analysis

For the quantitative scoring, the electronmicrographs of aortic endothelium were given a random number and scored blindly by three people. The pictures were scored from zero to three based on the level of structural changes seen in mitochondria, nucleus, vacuoles, lysosomes, Weibel-Palade bodies and intercellular connections. Score “zero” represents normal cell architecture, “one” slight change in cell structure, “two” moderate, and “three” represents severe injury of cell structure. The 3 independent scores were averaged together for each slide.
Differences were assigned between individual groups using Student’s t-test. Values were considered to be different significant when $p<0.05$. All data are expressed as mean ± SEM.

**Results**

**Blood pressure measurement**

The chronic stress did not affect the blood pressure in stressed normotensive Wistar rats when compared with non-stressed rats (111 ± 2 mm Hg vs. 113 ± 2 mm Hg). On the other hand, blood pressure was significantly increased in BHR exposed to chronic crowding comparing to basal values (130 ± 2 mm Hg vs. 140 ± 3 mm Hg, $p<0.05$).

**NO synthase activity**

The NO synthase activity was 7.75 ± 0.80 pmol/min/mg in aorta of control BHR. Chronic stress reduced the enzyme activity in stressed BHR (3.32 ± 0.52 pmol/min/mg, $p<0.01$). Crowding stress did not affect the NO synthase activity in aorta of Wistar rats comparing to non-stressed rats (5.25 ± 0.44 pmol/min/mg vs. 5.22 ± 0.79 pmol/min/mg).

**Transmission electron analysis**

The electronmicroscopic examination showed classic architecture of aortic endothelial cells in control normotensive Wistar rats and in BHR. However, in BHR the endothelial cells locally displayed slight till moderate subcellular injury (Fig.1) manifested by heterogeneous density of cell cytoplasm, presence of vacuoles, increased amount of lysosomes and Weibel-Palade bodies and electronlucent chromatin. Intercellular connections contained interdigitating complexes, overlapping clefts and end-to-end connections containing gap junctions and occasionally irregularly widened tight junctions.
The chronic stress resulted in structural alteration of endothelial cells of aorta of Wistar rats resembling those in non-stressed BHR (Fig.2). Ultrastructural analysis of aortic endothelial cells of stressed BHR showed more severe subcellular injury compared to control BHR (Fig.3). The cells were edematous, contained injured mitochondria, numerous vacuoles, and electronlucent chromatin. The integrity of the endothelial lining was locally destroyed. Quantitative scoring of subcellular alterations of endothelial cells is shown in Tab.1. In addition, smooth muscle cells located in subendothelial media also locally displayed subcellular injury (Fig.3).

**Discussion**

The vascular endothelium is an active, dynamic tissue that controls many important functions, including regulation of vascular tone, maintenance of blood circulation, fluidity, coagulation, and inflammatory responses (Galey and Webster 2004). Therefore, the impairment of endothelial structure may contribute to endothelial dysfunction that may initiate or contribute to changes in cell adhesion, lipid deposition, and other early steps leading to vascular diseases.

In the present study we have examined the effect of chronic social stress on the structure of aortic endothelial cells of rats with a genetic predisposition to hypertension. The most important finding of this study was that chronic crowding caused marked subcellular injury of endothelium in BHR and also slight one in stressed normotensive Wistar rats. Important is also the fact that even borderline hypertension was associated with focal subcellular damage of endothelial cells.

In our experiments we used a less traditional model of borderline hypertension with a normotensive mother and SHR father to prevent any effect of different maternal behavior and gestation environment of the hypertensive dams on blood pressure of their offspring.
(Woodworth et al. 1990, Porter et al. 2004). The model of borderline hypertension is appropriate for the investigation of stress-cardiovascular interactions since BHR are more sensitive to behavioral stress than normotensive and they do not develop age-dependent hypertension, as do SHR (Lawler et al. 1981). Basal blood pressure of BHR in the present work was in the range 130-150 mm Hg and was comparable to that reported by Woodworth et al. (1990). Impaired endothelial-dependent vascular relaxation was observed in offspring of hypertensive patients (Esch et al. 2002) and animals as well (Liu et al. 2002). Our results showing local damage of endothelial cells of the aorta of control BHR indicated that it might affect endothelial integrity and permeability and activate inflammatory processes, which can to initiate atherogenesis in a vessel (Schächinger et al. 1999). Since no such prominent structural alterations were demonstrated in the aortic endothelium of control Wistar rats, it is possible to eliminate the effect of processing on the cell structure in BHR. It can indicate genetic origin of alterations due to interaction of some peculiar genetic network present in the hypertensive subject including related genes of the renin-angiotensin-aldosterone system (Corvol et al. 1999), angiotensin-converting enzyme (Fornage et al. 1998), activation of extracellular dual phosphorylated (Thr202/tyr204) extracellular-signal activated protein kinases (ERK) (Barančík et al. 2007) as well as activation of sympathetic nervous system.

The endothelial cells display the remarkable heterogeneity in the vascular tree (Garlanda and Dejana 1997). Despite that several studies demonstrated similar structural injury of endothelial monolayer in resistant and conduit vessels of hypertensive animals (Kristek et al. 1997, Tribulová et al. 2000, Okruhlicová et al. 2005), indicating pronounced sensitivity of endothelium to increased blood pressure. Ultrastructural changes of endothelium were also observed in NO-deficient hypertension (Okruhlicová et al. 2000, Tribulová et al. 2000) accompanied with structural remodeling of vessel wall (Kristek et al. 1996, Cebová et al.
2006), associated with reduced NO production *per se* rather than with hypertension (Holécyová et al. 1996) and with attenuated vasodilatation.

Social chronic stress in a modern world represents an important risk factor for development of the cardiovascular disease. Its deleterious effects depend on the critical period of exposure, duration and type - as they all may alter functions of the basic autoregulatory stress response components: the hypothalamic-pituitary-adrenal axis and the sympathoadrenal medullar system activating renin-angiotensin-aldosterone system and sympathetic nervous system (McCarty and Gold 1996, Esch et al. 2002). Crowding evokes social stress reactions with prominent psychosocial components mimicking emotional state alterations (Bugajski 1999). Although crowding is a relatively weak stressor, chronic exposure may induce behavioral changes (Dubovický et al. 1999) and may affect the function of cardiovascular system, especially in individuals exhibiting higher responses to stressful conditions including genetic predisposition to hypertension. Our results demonstrated reduced activity of NO synthase in the aorta of stressed BHR. Ultrastructural injury of endothelial cell mitochondria of stressed BHR suggests that NO production may also be associated with an imbalance of defense antioxidative stress system that contributes to the reduction of NO in the cells. In addition, mitochondrial injury indicates also changes in intracellular ATP levels. The endothelial dysfunction is also accompanied with inflammation and procoagulation processes. In endothelial cells of stressed BHR we observed increased amount of Weibel-Palade bodies producing von Willenbrand factor that is known as a marker of adhesive, coagulative and thrombotic processes (Wannauette et al. 2006); furthermore there were seen vacuoles and lysosomes representing structural markers of cell degradation processes, and altered intercellular connections providing functional coordination and communication between endothelial cells (Isakson et al. 2006). All these subcellular alterations may indicate considerable changes in adhesive, and permeable properties of the endothelium that can
facilitate the transmigration of leukocytes into subendothelial area and accelerate progression of inflammation and atherosclerotic injury.

In conclusion: our results indicate that borderline hypertension is associated with the subcellular injury of aortic endothelial cells that may contribute to higher sensitivity of the endothelium to chronic stress and accelerate the arterial dysfunction.

Conflict of Interest
There is no conflict of interest.

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References


**Tab. 1.** Quantitative score of subcellular alterations in endothelial cells of the aorta.

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<tr>
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<th>Control</th>
<th>Stress</th>
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<tr>
<td>W</td>
<td>0.23±0.01</td>
<td>0.93±0.02</td>
</tr>
<tr>
<td>BHR</td>
<td>1.06±0.02</td>
<td>2.06±0.02*</td>
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*p<0.005, BHR vs. BHRs; †p<0.001, W vs. BHR.

W – Wistar rats, BHR – borderline hypertensive rats. Subcellular alterations included changes in mitochondria, nucleus, vacuoles, lysosomes, Weibel-Palade bodies and intercellular connections.
Figure Legends.

Fig.1.
Electronmicrographs showing subcellular injury of aortic endothelium of control borderline hypertensive rats. Magnification: A x16 000, B x15 000, C x11 000.

Fig.2.
Chronic stress-induced subcellular injury of aortic endothelium and smooth muscle cells in borderline hypertensive rats. Magnification: A x12 000, B x10 000, C x10 000, D x14 000.

Fig.3.
Electronmicrographs of endothelium and smooth muscle cells in control Wistar rats (A, B). Crowding stress-induced subcellular injury of aortic endothelium in stressed Wistar rats. Magnification: A x15 000, B x10 000, C x10 000.
