The study investigated the effect of chronic crowding stress on vascular function and nitric oxide (NO) production in rats with various family history of hypertension. Wistar (W), wBHR (offspring of W dams and spontaneously hypertensive sires), sBHR (offspring of spontaneously hypertensive dams and W sires) and spontaneously hypertensive rats (SHR) were used. Twelve-week-old males were divided into the control or crowded group for eight weeks. Basal blood pressure (BP, determined by tail-cuff plethysmography) of W, wBHR, sBHR and SHR rats was 112 ± 3, 129 ± 2, 135 ± 2 and 187 ± 3 mmHg, respectively. Crowding increased BP and reduced aortic NO synthase activity only in sBHR and SHR rats, without alterations in hypothalamic NO production. Acetylcholine-induced vasorelaxation of the femoral artery of stress-exposed rats was improved in W, unaltered in wBHR and sBHR and reduced in SHR. Crowding reduced serotonin-induced vasoconstriction in W and wBHR rats but had no effect in sBHR and SHR rats. In conclusion, the results suggest that crowded offspring of normotensive mothers were able to modify their vascular function in order to maintain BP at normal levels. On the other hand, offspring of hypertensive mothers were unable of effective adaptation of vascular function in stressful conditions resulting in gradual development of hypertension.

**Key words:** L-NAME, crowding, stress-induced hypertension, borderline hypertension, genetic predisposition

**INTRODUCTION**

Although the problem of stress-related hypertension has been addressed in several experimental studies, there are still conflicting data as to the nature of the
cardiovascular changes induced by stressors. In the acute stress models, increase of BP and heart rate are induced via sympathetic activation (1, 2). There is but little information on long-term stress, which is more relevant to the human situation and may result either in adaptation to the new environment or in exhaustion associated with behavioral, immune and cardiovascular disorders. Additionally, mechanisms leading to adaptation or to the development of hypertension during chronic stress are unclear and depend on many factors, such as nature, intensity and duration of stressor, animal strain, age, gender, etc. Moreover, several studies reported that genetic susceptibility may play a crucial role in the development of hypertension (3, 4) as well as in the ability to cope with stress (5).

Nitric oxide (NO) is one of the most potent vasodilating substances, which participates in modulation of vascular tone and thus in BP regulation (6, 7). The finding that acute stress may enhance NO production in the neuroendocrine system of normotensive rats (8, 9) suggests that NO may represent one of the stress-limiting systems (10). To investigate the effect of chronic stress, we used social stress produced by crowding because it evokes social-stress reactions with prominent psychosocial components mimicking emotional state alterations (11). Although crowding is a relatively mild stressor, it affects signal transduction in hypothalamic-pituitary-adrenal responses (12 - 14). In humans, crowded residents had higher levels of urinary catecholamines and greater increases in blood pressure and heart rate during performance of a challenging task than non-crowded residents (15), suggesting a deleterious effect of chronic crowding on cardiovascular regulation. However the effect of chronic social stress on central and vascular NO synthesis and its relation to regulation of vascular function and blood pressure depending on genetic predisposition to hypertension is unclear.

Thus, the purpose of this study was to investigate vascular function and NO production in chronic crowding-stress-exposed rats with various family history of hypertension. We used three cardiovascular phenotypes of rats: normotensive Wistar (W) rats, borderline hypertensive (BHR) and spontaneously hypertensive (SHR) rats. Moreover, BHR were produced as offspring of either normotensive or spontaneously hypertensive dams, to elucidate the influence of the mother on stress-related cardiovascular regulation of litters in adulthood.

METHODS

Animals

All rats used in the study were born in our animal facility in order to maintain the same environmental background of all objects. BHR were F1 offspring of either SHR dams and normotensive Wistar sires (sBHR) or Wistar dams and SHR sires (wBHR). The rats were housed at 22-24°C on a 12:12-h dark-light cycle and maintained on a standard pellet diet and tap water ad
libitum. All procedures used were approved by the State Veterinary and Food Administration of the Slovak Republic.

**Stress model**

At the beginning of the experiment, adult male rats, 12 weeks old, were randomly divided into control or stressed groups. Controls were kept in groups of 4 rats/cage (35/55/20 cm). Rats exposed to crowding stress were kept in groups of 5 rats/cage (25/40/15 cm) for eight weeks. All rats had food and water *ad libitum*.

One week before experimentation, the rats were handled and accustomed to the tail-cuff procedure of blood pressure (PB) and heart rate (HR) recording. BP and HR were determined before experiment (basal) and after the 1st, 3rd, 6th and 8th week of experiment. Body mass (BM) was recorded on the same days. After 8 weeks of experiment, the rats were killed by decapitation after a brief diethyl ether anesthesia and body mass as well as the wet mass of the adrenal glands (AG) were determined for calculation of their relative mass (AG/BM).

**NO synthase activity**

NO synthase activity was measured in the homogenate of the thoracic aorta and hypothalamus by determination of \[^3H\]-L-citrulline formation from \[^3H\]-L-arginine (Amersham, UK), as described previously (16). NO synthase activity was expressed as pmol/min/mg of proteins.

**Vascular reactivity**

Vascular reactivity was investigated in rings of the femoral artery using Mulvany-Halpern’s myograph as described elsewhere (17). Arteries were pre-contracted with phenylephrine (PE, 10^{-4} mol/l) and acetylcholine (ACh) was applied in cumulative manner (10^{-9}–10^{-5} mol/l). When the concentration-relaxation curve was completed, the drugs were washed-out (with 4 × 10 ml of Krebs-Ringer solution) and the same experiment was repeated after 20-min preincubation with the nitric oxide synthase inhibitor N\textsubscript{G}-nitro-L-arginine methyl ester (L-NAME, 1 µmol/l) in the bath medium.

The dose-response curve to serotonin (5-hydroxytryptamine, 5-HT) was constructed in a cumulative manner (10^{-9}–10^{-5} mol/l) in other segments of the femoral artery. On reaching the maximal constriction, the chamber solution was completed, and the chamber was washed with fresh Krebs-Ringer solution (37°C) several times. After a 40-min equilibration period the dose-response curve to noradrenaline (NA) was constructed in a cumulative manner (10^{-9}–10^{-5} mol/l).

The relaxation of the femoral artery was expressed as the percentage of maximal pre-contraction. The contractile responses of the femoral arteries were reported as active wall tension (mN/mm). The effect of stress in each rat strain was also expressed as the average value of relaxation and constriction (effect of stress/strain interaction), which was calculated from the individual dose-response curves.

**Statistical analysis**

All results are presented as mean ± SEM. Blood pressure and body mass were analyzed using three-way ANOVA (strain x group x week of experiment). NO synthase activity and relative mass of the adrenal glands were analyzed using two-way ANOVA (strain x group). Vasorelaxation was analyzed using four-way ANOVA (strain x group x concentration of ACh x L-NAME). Vasoconstriction was analyzed using three-way ANOVA (strain x group x concentration of agonist).
All analyses were followed by Duncan’s post-hoc test. Values were considered to differ significantly when \( p < 0.05 \).

**RESULTS**

**Basic parameters**

Body mass of Wistar, wBHR, sBHR and SHR rats before experiment was 281 ± 6 g (\( n = 36 \)), 309 ± 5 g (\( n = 20, p < 0.01 \) vs. W), 287 ± 5 g (\( n = 30, p < 0.03 \) vs. wBHR) and 243 ± 3 g (\( n = 30, p < 0.01 \) vs. W), respectively and crowding failed to alter the body mass gain (*Table 1*).

Basal blood pressure of Wistar, wBHR, sBHR and SHR rats before experiment was 112 ± 3 mm Hg (\( n = 36 \)), 129 ± 2 mm Hg (\( n = 20, p < 0.0001 \) vs. W), 135 ± 2 mm Hg (\( n = 30, p < 0.02 \) vs. wBHR) and 187 ± 3 mm Hg (\( n = 30, p < 0.0001 \) vs. all), respectively. Crowding increased BP significantly (\( F(1, 530) = 20.3, p < 0.0001 \), main effect of stress). Crowding/strain interaction revealed significant differences among the groups (\( F(3, 530) = 3.6, p < 0.02 \), *Table 1*), with crowding resulting in a significant increase of BP in sBHR and SHR vs. control and no alterations of BP in W and wBHR rats. In wBHR rats, 6-week crowding elevated BP vs. their pre-stress value (*Fig. 1*) but no differences were observed at the end of experiment.

Basal heart rate of Wistar, wBHR, sBHR and SHR rats before experiment was 399 ± 7 bpm (\( n = 16 \)), 405 ± 8 bpm (\( n = 16 \)), 408 ± 12 bpm (\( n = 20 \)) and 443 ± 10 bpm (\( n = 24, p < 0.05 \) vs. all), respectively and crowding had no significant effect on HR in any strain studied (*Table 1*).

Relative adrenal gland mass (AG/BM) was determined as an indirect marker of stress exposure. Significant increase of the relative adrenal gland mass was observed only in sBHR (*Table 1*).

*Table 1.* Body mass (BM), blood pressure (BP), heart rate (HR) and relative adrenal gland mass (AG/BM) in control and stress-exposed rats (stress/strain interaction).

<table>
<thead>
<tr>
<th></th>
<th>Wistar</th>
<th>wBHR</th>
<th>sBHR</th>
<th>SHR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n</strong></td>
<td>75-120</td>
<td>75-105</td>
<td>40-55</td>
<td>12-16</td>
</tr>
<tr>
<td><strong>BM (g)</strong></td>
<td>309 ± 4</td>
<td>311 ± 5</td>
<td>411 ± 6</td>
<td>7.4 ± 0.2</td>
</tr>
<tr>
<td><strong>BP (mm Hg)</strong></td>
<td>315 ± 8</td>
<td>332 ± 5</td>
<td>402 ± 8</td>
<td>7.2 ± 0.2</td>
</tr>
<tr>
<td><strong>HR (bpm)</strong></td>
<td>342 ± 6</td>
<td>316 ± 5</td>
<td>420 ± 5</td>
<td>6.1 ± 0.1</td>
</tr>
<tr>
<td><strong>AG/BM (mg/100g)</strong></td>
<td>320 ± 3</td>
<td>132 ± 3</td>
<td>416 ± 8</td>
<td>6.5 ± 0.1</td>
</tr>
</tbody>
</table>

**Abbreviations:** wBHR - Wistar-mothered borderline hypertensive rats, sBHR - SHR-mothered borderline hypertensive rats, SHR – spontaneously hypertensive rats. Given are the results of statistical analysis for factors stress and strain. Data are expressed as mean ± SEM; *=p<0.05 vs. control of the same strain, ”*p<0.01 vs. Wistar of the same group, #*p<0.05 vs. wBHR of the same group, *p<0.01 vs. all Wistar, wBHR and sBHR of the same group.
Nitric oxide synthase activity

Nitric oxide synthase activity in the aorta \( (n = 6-7 \text{ per group}) \) of Wistar rats was lower than in any strain with a positive family history of hypertension (Fig. 2). Crowding failed to affect NO synthase activity in the aorta of W and wBHR rats (Fig. 2). However in both sBHR and SHR crowding significantly reduced NO synthase activity in the aorta by 30\% (\( p < 0.05 \)) and 51\% (\( p < 0.008 \)), respectively.

Lower basal NO synthase activity was observed in the hypothalamus of wBHR when compared to W rats (Fig. 2) and crowding failed to alter hypothalamic NO production in all strains.

Vascular function

Vascular function (ACh-induced vasorelaxation, NA-induced vasoconstriction and serotonin-induced vasoconstriction) were investigated in 6-7 rats per group. ANOVA revealed significant strain-dependent differences in ACh-induced relaxation (\( F(3,711) = 13.3, p < 0.00001 \), main effect of strain). Crowding alone had no effect on ACh-induced relaxation (59.1 ± 2 \% in controls vs. 59.9 ± 2 \% in crowded rats, main effect of crowding). However, significant differences in ACh-induced relaxation were observed in strain/crowding interaction (\( F(3,711) = 7.9, p < 0.0001 \)) when stress elevated vasorelaxation in W rats, had no effect in wBHR and sBHR and reduced it in SHR rats (Fig. 3 and 4). Pretreatment of the arterial segments with L-NAME significantly reduced ACh-induced relaxation.
L-NAME blunted stress-induced elevation of vasorelaxation in W rats, had no effect in the either BHR strains and reduced relaxation in SHR (Fig. 3 and 4).

Serotonin-induced vasoconstriction was not different among the strains and crowding significantly reduced it (F(1,450) = 16.4, p<0.0001, main effect of crowding). Interaction of strain and crowding revealed significant decrease of serotonin-induced vasoconstriction in crowded rats with normotensive mother, i.e. in W and wBHR rats (Table 2, Fig. 5), yet no alterations were recorded in SHR-mothered rats.

(F(1,711) = 32.6, p<0.0001). L-NAME blunted stress-induced elevation of vasorelaxation in W rats, had no effect in the either BHR strains and reduced relaxation in SHR (Fig. 3 and 4).

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Table 2. Noradrenaline- and serotonin-induced vasoconstriction of the femoral artery in control and stress-exposed rats (stress/strain interaction).

<table>
<thead>
<tr>
<th></th>
<th>Wistar</th>
<th>wBHR</th>
<th>sBHR</th>
<th>SHR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>NA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stress</td>
<td>1.54±0.2</td>
<td>0.40±0.1</td>
<td>0.56±0.1</td>
<td>1.52±0.2</td>
</tr>
<tr>
<td>5-HT</td>
<td>9.6±1.0</td>
<td>6.4±1.1</td>
<td>6.2±0.9</td>
<td>6.8±0.9</td>
</tr>
</tbody>
</table>

Abbreviations: wBHR - Wistar-mothered borderline hypertensive rats, sBHR - SHR-mothered borderline hypertensive rats, SHR – spontaneously hypertensive rats; NA – noradrenaline, 5-HT - serotonin. Given are the results of statistical analysis for factors stress and strain. Data are expressed as mean ± SEM; n = 54-63, *p<0.05 vs. control of the same strain, †p<0.05 vs. Wistar of the same group, ‡p<0.05 vs. all Wistar, wBHR and sBHR of the same group.
In NA-induced vasoconstriction ANOVA revealed significant strain-dependent differences \( F(3, 378) = 3.3, p<0.0001 \), main effect of strain) with highest values found in SHR \( 4.24 \pm 0.35 \text{ mN/mm}, p<0.0001 \) and significant reduction found in wBHR \( 0.61 \pm 0.11 \text{ mN/mm}, p<0.0001 \) and sBHR \( 1.02 \pm 0.12 \text{ mN/mm}, p<0.0003 \) vs. W \( 2.03 \pm 0.26 \text{ mN/mm} \). Crowding significantly increased vasoconstriction vs. control rats \( F(1, 378) = 31.1, p<0.0001 \), main effect of crowding) and significant differences were observed also in strain/crowding interaction \( F(3, 378) = 13.08, P<0.03 \). Stress elevated NA-induced responses in
The most important finding of this study was that a relatively mild stressor, chronic crowding, affected vascular function and NO production of rats depending on the history of hypertension in their parents. This was seen mainly in differences in endothelium-dependent relaxation: crowding improved ACh-induced relaxation in normotensive rats, had no effect in rats with one hypertensive parent and attenuated vasorelaxation in rats with two hypertensive parents. Differences were also observed in serotonin-induced vasoconstriction. Reduced serotonin-induced vasoconstriction was observed in crowded offspring of normotensive mothers, while no changes occurred in offspring of hypertensive mothers. Additionally, reduced vascular NO production was observed only in stress-exposed offspring of SHR dams. Altogether, these alterations in vascular function and NO production resulted in elevation of blood pressure in offspring of hypertensive mothers while only transient or no changes in BP were observed in offspring of normotensive mothers.

It is well known that neurogenic mechanisms, mainly sympathetic activation, play a significant role in cardiovascular responses in stress and increase of BP is
associated with tachycardia (18, 19) or tachycardia may be present without alterations in BP (20). On the other hand, elevation of BP without changes in HR, as observed in our study, indicates rather the involvement of peripheral vascular regulatory mechanisms. This is supported by the finding of unchanged hypothalamic NO synthesis in crowded rats, suggesting that the presented stress paradigm does not affect central NO production. This, however, does not exclude transient alterations in central NO production in the course of crowding, similarly as observed in restraint-exposed rats (21).

The involvement of vascular mechanisms in elevation of resting BP was observed in animal as well as in human studies. Sustained elevation of BP was recorded in rats that responded to chronic unconditioned stress stimulus via elevated systemic vascular resistance (22). In humans, individuals with positive

Fig 5. Effect of chronic crowding on serotonin-induced vasoconstriction of the femoral artery. Abbreviations: 5-HT – serotonin, wBHR - Wistar-mothered borderline hypertensive rats, sBHR - SHR-mothered borderline hypertensive rats, SHR – spontaneously hypertensive rats. Results are mean ± SEM. * p<0.05 vs. control
parental history of hypertension responded to mental stress by elevation of BP, also induced primarily by an increase in total vascular resistance with negligible changes in HR (23). Similarly in the present study, crowding-induced changes in BP, without changes in HR, were observed only in rats with at least one hypertensive parent. In Wistar-mothered BHR, BP was gradually increasing until the sixth week of experiment. A similar increase of BP associated with altered vascular function in wBHR was observed also in our previous study (24). However, the extension of crowding to eight weeks resulted in adaptation of their pressor response as well as in normalization of vascular function. In contrast, sBHR did not adapt and they developed the greatest augmentation of BP of all groups investigated. The results suggest that offspring of hypertensive mothers were not able to cope with chronic crowding as effectively as did offspring of normotensive mothers. This may be associated either with altered intrauterine development (25) or with neonatal stress produced by different maternal behaviour of the hypertensive mothers (26, 27), which may alter basal vascular function and sensitize the stress response of the litters. Indeed, we observed significant strain-dependent differences in vascular function of the femoral artery already in the control conditions. Surprisingly, however, elevated vasorelaxation of the femoral artery was found in control sBHR and SHR rats, in contrast to previous studies showing reduced vasorelaxation of various arteries in SHR (28, 29). On the other hand, our finding of improved ACh-induced vasorelaxation of the femoral artery in control SHR is in agreement with observations of Konishi and Su (30) who found improved vasodilatation in the femoral artery of SHR concurrently with impaired vasodilatation in the aorta. Similarly, the magnitude of the ACh-induced vasodilatation was greater in the mesenteric arteries of SHR than in normotensive rats (31). In BHR, vasorelaxation of the thoracic aorta was higher than in normotensive controls, while no differences were observed in the mesenteric artery (32, 33). These findings suggest that endothelial dysfunction in hypertension is not a universal finding and vascular function may be affected differently in various parts of the vascular tree.

Concerning endothelium-dependent vasorelaxation in stress, there are studies showing that stress may induce endothelial dysfunction. In rats, acute immobilization led to endothelium, suggesting alteration of the endothelial function (37). Similarly, chronic social conflict increased the rate of endothelial cell damage (38) and reduced vasorelaxation of the iliac artery (39) in primates. On the other hand, increased vasodilator responses of the aorta were observed in mice exposed to chronic social stress (40). Similarly, we observed improved endothelium-dependent ACh-induced relaxation of the femoral artery in both crowding-exposed W rats in this study as well as in Wistar-Kyoto rats (17). In contrast to normotensive rat strains, reduced vasorelaxation was observed in crowded SHR while no alterations were seen in either of the borderline hypertensive strains. NO synthase inhibitor L-NAME reduced the elevation of the endothelium-dependent vasorelaxation in crowded W rats to the control level,
suggesting that augmentation of vasorelaxation in stressed W rats was NO-dependent. The greatest inhibitory effect of L-NAME was observed in SHR, in agreement with our recent observation of the greatest L-NAME-sensitive component of ACh-induced relaxation in spontaneously hypertensive rats (41).

The finding of unchanged endothelium-dependent vasorelaxation in crowded sBHR concurrently with their reduced vascular NO synthesis is surprising. We hypothesize that unaltered endothelium-dependent relaxation in crowded sBHR may result from activation of other ACh-induced vasorelaxing mechanisms, such as hyperpolarization (42), or simply from the fact that NO synthesis in stressed sBHR was still comparable to that in control Wistar rats and thus probably sufficient for normal vasodilatation. Moreover, the given dose of L-NAME had no effect on vasorelaxation in borderline hypertensive rats. From this point of view, elevated basal vascular NO synthesis in rats with a positive family history of hypertension may be considered an adapting mechanism, preventing them from excessive BP elevation in stressful conditions and/or during modest reduction of NO synthesis.

Regarding vasoconstriction, hyporeactivity to NA was observed in both control WBHR and sBHR rats in contrast to SHR rats. Similarly, reduction of α-adrenoceptor mediated vasoconstriction was found in the mesenteric arteries of sBHR rats using phenylephrine (32). Reduced NA-induced vasoconstriction in borderline hypertensive rats may result either from functional down-regulation of α-adrenoceptors or from the elevated basal NO synthesis, because NO is known to counterbalance the effect of sympathetic stimulation on the peripheral as well as central level (34, 35). However, not even elevated basal vascular NO synthesis resulted in attenuation of NA-induced vasoconstriction in SHR rats, supposedly due to their altered vascular wall structure (36).

It is interesting that although low basal NA-induced vasoconstriction was observed in both BHR strains, crowding had the greatest effect on vasoconstriction in sBHR rats, while only a mild effect was observed in WBHR. This is in agreement with the observation of different effects of crowding on BP in WBHR and sBHR rats, supporting the idea of impaired cardiovascular adaptation of offspring of hypertensive mothers.

Since stress stimulates release of serotonin from platelets (43), this can also significantly contribute to the final vasoactivity of the arteries and thus to regulation of blood pressure. The effects of serotonin on regional hemodynamics are known to vary according to the vascular bed and even in the same vascular bed (44). In this study, crowding attenuated serotonin-induced vasoconstriction in both Wistar-mothered strains, which was not observed in offspring of SHR dams. This suggests that chronic stress may differently affect distribution and/or affinity of vasodilating “5-HT1-like” and vasoconstrictive 5-HT2 receptors in the femoral artery of offspring of normotensive and hypertensive mothers. Thus blunted serotonin-induced vasoconstriction may be another mechanism protecting Wistar-mothered rats from social stress-induced hypertension. However other
factors, such as altered oxidative status (45), may play a role in modulation of vascular function in stress. Recently, Miyashita et al. (46) have shown that 7-day crowding elevated urinary excretion of oxidative metabolites of bilirubin in mice. However, only slightly elevated levels of oxidative metabolites were observed after 30 days of crowding, suggesting adaptation during chronic stress exposure (46). Thus, more experiments are needed to elucidate the exact mechanism of vascular response and adaptation in course of chronic stress in individual cardiovascular phenotypes.

In conclusion, the results suggest that chronic social stress produced by crowding affected differently vascular function and NO synthase activity of rats depending on their family history of hypertension. Stress-exposed rats with negative family history of hypertension were able to cope with chronic stress by improvement of vasorelaxation and reduction of serotonin-induced vasoconstriction without significant alteration in vascular NO production. Reduced serotonin-induced vasoconstriction and normal vasodilatation and NO production were observed also in borderline hypertensive offspring of normotensive mothers. On the other hand, chronic stress led to the reduction of vascular NO synthesis in offspring of spontaneously hypertensive mothers, which was associated with accentuation of NA-induced and unchanged serotonin-induced vasoconstriction in both sBHR and SHR. Moreover, reduced vasodilatation was observed in stress-exposed rats with two hypertensive parents. On balance then, the hypertensive phenotype of the mother represents a risk factor for the development of chronic social-stress-induced hypertension since offspring of hypertensive mothers were unable of effective adaptation of their vascular function in stressful conditions.

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REFERENCES


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Author’s address: Iveta Bernatova, Ph.D., Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, Sienkiewiczova 1, 813 71 Bratislava, Slovak Republic. Tel.: +421-2-52926336, fax: +421-2-52968516; e-mail: Iveta.Bernatova@savba.sk