

## Crowding-Induced Alterations in Vascular System of Wistar-Kyoto Rats: Role of Nitric Oxide

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### Summary

The aim of this study was to determine the effect of chronic crowding on the cardiovascular system of Wistar-Kyoto (WKY) rats. Rats were randomly divided into the control (480 cm<sup>2</sup> per rat) or crowded (200 cm<sup>2</sup> per rat) group for eight weeks. Body weight, blood pressure (BP), heart rate and plasma nitrate/nitrite levels of the crowded rats were not different from controls at the end of the experiment. Plasma corticosterone exhibited an increasing trend (5.7±1.8 vs. 12.6±3.7 ng/ml, p=0.08) while blood glucose was significantly reduced in the crowded rats in comparison with the controls. Nitric oxide (NO) synthase activity and nitrate/nitrite levels of the crowded rats were significantly elevated in the aorta by ~80 % and ~20 %, respectively, but unchanged in the left ventricle. Moreover, acetylcholine-induced relaxation was significantly increased in the crowded rats in both the femoral artery (61±5 % vs. 76±5 %, p<0.001) and mesenteric artery (51±6 % vs. 72±7 %, p<0.001). In conclusion, results suggest that chronic crowding may increase vasorelaxation and vascular NO production in normotensive rats. This may be considered as an adapting mechanism preventing the development of the stress-related elevation of BP. Additionally, results also suggest caution in the housing of rats because an inappropriate crowding may affect results of the experiment significantly.

### Key words

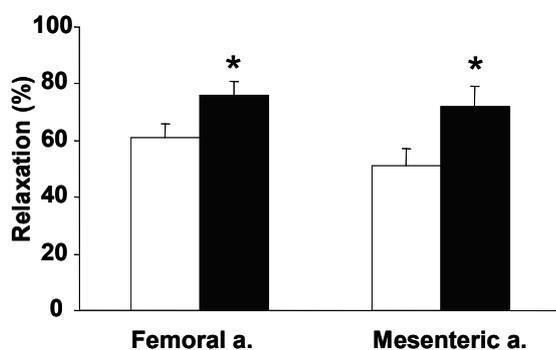
Social stress • Vasorelaxation • Corticosterone • Adaptation

Chronic crowding typically evokes social-stress reactions with prominent psychosocial components mimicking emotional state alterations (Bugajski 1999). Although the crowding is a relatively mild stressor, it affects signal transduction in hypothalamic-pituitary-adrenal responses in rats (Bugajski 1999). In humans, crowded residents had higher levels of urinary

catecholamines and greater increases of blood pressure and heart rate during the performance of a challenging task than the uncrowded ones (Fleming *et al.* 1987), suggesting a deleterious effect of the chronic crowding on cardiovascular regulations. Nevertheless, there are still conflicting data as to the nature of cardiovascular changes induced by stress in normotensive animals (Andrews *et*

al. 2003, Grippo *et al.* 2002). Thus, we have investigated the effect of the chronic crowding on blood pressure, nitric oxide (NO) production and vascular function in normotensive WKY rats.

Twelve-week-old males WKY rats (born in our approved animal facility) were divided into control (480 cm<sup>2</sup>/rat, 4 rats/cage) and crowded (200 cm<sup>2</sup>/rat, 5 rats/cage) groups for eight weeks. Body weight (BW), blood pressure (BP), heart rate (HR), blood glucose, plasma corticosterone and plasma nitrate/nitrite (pNit) levels were determined at the end of the experiment. BP and HR were determined by tail-cuff. Rats were killed by decapitation after a brief CO<sub>2</sub> anesthesia between 7.30-9.30 a.m. Glucose was determined by the Ascensia ENTRUST<sup>®</sup> blood glucose meter in blood taken from the end of the tail. Plasma corticosterone and pNit were determined from the trunk blood. Plasma corticosterone was determined by RIA (DRG Instruments, Germany) and pNit as well as nitrate/nitrite levels in the aorta and left ventricle were determined by the Griess reaction (Green *et al.* 1982). NO synthase activity (NOS, determined in homogenates of the aorta and the left ventricle) and the acetylcholine (ACh)-induced relaxation of the femoral and first branches of the superior mesenteric artery were measured as described previously (Púzserová *et al.* 2006). All procedures used in this study were approved by the State Veterinary and Food Administration of the Slovak Republic. Results are presented as mean  $\pm$  SEM. BP, HR, BW and vasorelaxation were analyzed using two-way ANOVA and Duncan's test. All other data were analyzed by Student t-test. Values were considered to differ significantly if  $p < 0.05$ .



**Fig. 1.** Acetylcholine-induced relaxation of the femoral and the first branches of the superior mesenteric artery in control (white bar) and crowded (black bar) WKY rats. Values were calculated as the average values of the individual acetylcholine-induced dose-response curves ( $10^{-5}$ - $10^{-9}$  mol/l) in phenylephrine-precontracted vascular rings in isometric conditions. Results are means  $\pm$  S.E.M. \* $p < 0.001$  vs. control.

**Table 1.** Effect of crowding on selected physiological and metabolic parameters of WKY rats.

	n	Control	Crowding
Body weight (g)	7	390 $\pm$ 10	376 $\pm$ 11
Blood pressure (mm Hg)	7	110 $\pm$ 3	111 $\pm$ 2
Heart rate (bpm)	7	335 $\pm$ 15	344 $\pm$ 10
Blood glucose (mmol/l)	5	6.5 $\pm$ 0.2	4.9 $\pm$ 0.2*
Plasma corticosterone (ng/ml)	5	5.7 $\pm$ 1.8	12.6 $\pm$ 3.7
Plasma nitrite/nitrate (nmol/ml)	5	84 $\pm$ 2	76 $\pm$ 5
NOS - Aorta (pmol/min/mg)	6	2.6 $\pm$ 0.2	4.7 $\pm$ 0.5*
NOS - LV (pmol/min/mg)	6	2.1 $\pm$ 0.4	2.2 $\pm$ 0.5
Nitrate/nitrite - Aorta (nmol/mg)	5	6.7 $\pm$ 0.2	7.9 $\pm$ 0.3*
Nitrate/nitrite - LV (nmol/mg)	5	5.4 $\pm$ 0.5	5.8 $\pm$ 0.2

NOS – nitric oxide synthase activity, LV – left ventricle. Results are means  $\pm$  SEM. \* $p < 0.05$  vs. control.

As described in the Table 1, concentrations of plasma corticosterone in the crowded rats exhibited an increasing trend ( $p = 0.08$  vs. control) and blood glucose was significantly reduced, suggesting a mild stress response. Regarding the cardiovascular response, a given paradigm of the chronic crowding failed to affect BP, HR and plasma nitrate/nitrite of WKY rats. Additionally, no changes were seen in NOS activity and nitrate/nitrite level in the left ventricle. However, in the aorta, NOS activity and nitrate/nitrite level were significantly elevated in the crowded rats. Additionally, the crowding elevated the values of ACh-induced relaxation of both the femoral artery and the mesenteric artery (Fig. 1). This increase was blunted by the NO synthase inhibitor L-NAME (Púzserová *et al.* 2006). Thus, despite no effect on blood pressure and heart rate, this study has shown that the chronic crowding may affect the glucose metabolism, vascular NO production and vascular function of WKY rats. Similar crowding-induced changes had been previously observed in Wistar rats (Bernátová *et al.* 2006, 2007). On the other hand, elevated blood

pressure, reduced NO production and unaltered or reduced vasorelaxation were observed in rats with a positive family history of hypertension, i.e. in borderline and spontaneously hypertensive rats (Bernátová *et al.* 2007). These observations suggest that elevated NO production in normotensive rats may be considered to be an adapting mechanism preventing the development of the stress-induced elevation of blood pressure. This supports the idea that L-arginine/NO system may act as one of the stress-limiting systems in organism and failure of this system may result in development of a stress-related hypertension.

In conclusion, results suggest that 8-week crowding supposedly does not represent a significant risk

factor for the development of stress-related hypertension in normotensive rats because of their ability to adapt to stressful conditions. However, results also suggest a caution in housing of rats, especially of those involved in cardiovascular studies because inappropriate housing in crowded cages may significantly affect the results of the experiment, regardless the absence of blood pressure changes.

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