

## Rapid Communication

---

T. ZERA, M. UFNAL, E. SZCZEPANSKA-SADOWSKA

### CENTRAL TNF- $\alpha$ ELEVATES BLOOD PRESSURE AND SENSITIZES TO CENTRAL PRESSOR ACTION OF ANGIOTENSIN II IN THE INFARCTED RATS

Department of Experimental and Clinical Physiology, Medical University of Warsaw, Poland

In patients with chronic heart failure (CHF) concentration of TNF- $\alpha$  is elevated. Enhanced synthesis of TNF- $\alpha$  was also found in the hypothalamus of rats shortly after induction of the myocardial infarct. Available evidence indicates that TNF- $\alpha$  increases sympathetic activity and enhances function of the renin-angiotensin-aldosterone system in peripheral tissues. The role of TNF- $\alpha$  in regulation of the cardiovascular system and its interactions with brain angiotensin II (ANGII) in CHF was evaluated in the following study. Fourteen Sprague-Dawley rats underwent left coronary artery ligation, implantation of lateral cerebral ventricle cannula and insertion of femoral artery catheter. Post-infarct CHF was confirmed by increased left ventricle end-diastolic pressure. Mean arterial blood pressure (MABP) and heart rate (HR) were recorded during 60 min of intracerebroventricular (*i.c.v.*) infusion of 0.9% NaCl (5  $\mu$ l/hr) (control group, n = 7) or TNF- $\alpha$  (100 ng/5 $\mu$ l/hr) (experimental group, n = 7). This was followed by *i.c.v.* injection of subpressor dose of ANGI (5 ng/2  $\mu$ l/30 sec) and measurements were continued for 20 min. Infusion of TNF- $\alpha$  resulted in the increase of MABP without changes in HR. Administration of ANGI elicited significantly greater increase of MABP in rats pretreated with TNF- $\alpha$ . Present results indicate that TNF- $\alpha$  increases MABP in CHF and sensitizes to pressor effect of centrally administered ANGI.

Key words: TNF- $\alpha$ , ANG II, heart failure

#### INTRODUCTION

Tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) is a member of a vast family of pro-inflammatory cytokines. Increased plasma concentration of this cytokine is a common finding in patients with a chronic heart failure (CHF) (1). Enhanced

synthesis of TNF- $\alpha$  in the hypothalamus of rats has been also found shortly after induction of the myocardial infarct (2, 3). TNF- $\alpha$  has been shown to be involved in generating the oxidative stress and sympathetic activation during the post-infarct heart failure (4). Some studies indicate that TNF- $\alpha$  may also “wind-up” the renin-angiotensin-aldosterone system in the peripheral tissues, in particular in the heart, kidney and arteries (5, 6, 7), however the role of centrally acting TNF- $\alpha$  in regulation of the cardiovascular system and its interaction with angiotensin II (ANGII) is not yet well recognised. We have previously found that intracerebroventricular (*i.c.v.*) administration of TNF- $\alpha$  does not elevate the resting mean arterial blood pressure but it causes sensitisation to the central pressor action of angiotensin II (8). The aim of the present study was to determine the role of entral TNF- $\alpha$  in regulation of the cardiovascular system during the post-infarct CHF and to elucidate whether there is an interaction of TNF- $\alpha$  with the brain angiotensin system in this respect.

#### MATERIAL AND METHODS

The experiments were performed on 14 male Sprague Dawley rats (age 12-14 weeks) in accordance with the principles of the NIH Guide for the Care and Use of Laboratory Animals and the experimental protocol approved by the Ethical Committee of the Medical University of Warsaw. All animals underwent ligation of the coronary artery in order to induce the heart failure (9). After four weeks each animal was implanted with the lateral cerebral ventricle cannula according to the following co-ordinates: 1.2 mm caudal to the bregma, 1.8 mm lateral to the sagittal suture. The tip of the cannula was placed 3.5-4.5 mm below the surface of the skull. One week later a polyethylene catheter was implanted through the femoral artery into the aorta below the branching of the renal arteries. All surgical procedures were performed under general anaesthesia with ketamine (100 mg/kg *i.p.*) and xylazine (10 mg/kg *i.p.*).

#### *Experimental protocol*

After the recovery period of 24-48 hours mean arterial blood pressure (MABP) and heart rate (HR) were recorded online with BIOPAC system and saved on the PC hard drive. After stabilisation of the haemodynamic parameters baseline MABP and HR were recorded for 10 min before the onset of the intracerebroventricular (*i.c.v.*) infusion and during 60 min of *i.c.v.* infusion of either vehicle (0.9% NaCl; 5  $\mu$ l/hr; control group, n = 7) or TNF- $\alpha$  (Sigma-Aldrich; 100 ng/5 $\mu$ l/hr; experimental group, n = 7). This was followed by *i.c.v.* injection of a subpressor dose of ANGI (Sigma-Aldrich) (5 ng/2 $\mu$ l/30 sec) and MABP and HR measurements were continued during the next 20 min. At the end of the experiment presence of the heart failure was verified by direct measurements of the end-diastolic left ventricle pressure (EDLVP) with the Millar catheter and the data from the rats with EDLVP exceeding 15 mm Hg were included into the statistical analysis.

All results are expressed as means  $\pm$  SEM. Averaged values of 5 min time intervals were used in the analysis of haemodynamic parameters during TNF- $\alpha$  and saline infusions and 1 min intervals in the analysis of the data obtained after injection of ANGI. Two way ANOVA for repeated measurements was used for evaluation of differences between groups, and one-way ANOVA for repeated measurements and Tukey test were performed for comparisons within the same group. Post-hoc comparisons of the corresponding time points between the groups were made using Bonferroni correction for Student t-test.

## RESULTS

Baseline MABP and HR values in the control and the experimental groups did not differ and amounted to: control: MABP =  $109.59 \pm 2.92$ , HR =  $334.81 \pm 7.17$ ; experimental: MABP =  $107.30 \pm 2.81$ , HR =  $339.13 \pm 4.11$ . Infusion of TNF- $\alpha$  resulted in significant increase of MABP (Fig. 1, panel A) that was not associated with significant changes of HR. Infusion of saline did not influence significantly the haemodynamic parameters. Changes of MABP in the group receiving *i.c.v.* infusion of TNF- $\alpha$  were significantly different from those in the group receiving vehicle (Fig. 1, panel A). *i.c.v.* injection of ANGII elicited significant increase in MABP both in the control and the experimental group, however the pressor effect was significantly greater and lasted longer in the group pretreated with TNF- $\alpha$  than in the group pretreated with the vehicle (Fig. 1, panel B). Heart rate tended to increase after administration of ANGII but the changes were not significant either in the control or in the experimental group (data not shown).

## DISCUSSION

Numerous lines of evidence suggest relevance of TNF- $\alpha$  to regulation of the cardiovascular functions, especially during the post-infarct state (1-3, 12,13). TNF- $\alpha$  is synthesised in the hypothalamus, particularly in the paraventricular nucleus upon induction of the myocardial infarct (2,3). Its actions in the hypothalamus include generation of the radical oxygen species and stimulation of the sympathetic outflow (4). Blocking of central mineralocorticoid receptors is thought to reduce the sympathetic activation *via* suppression of TNF- $\alpha$  expression (10,11). There are also findings showing that TNF- $\alpha$  stimulates renin-

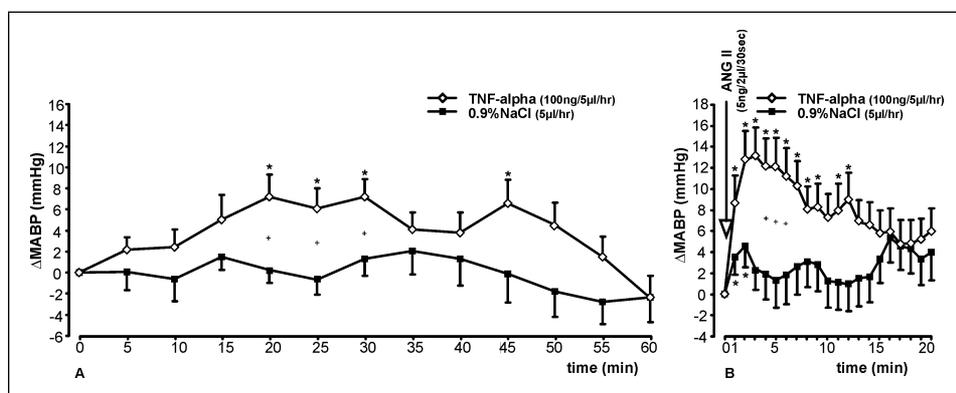


Fig. 1. Changes of MABP during infusion of TNF- $\alpha$  (100 ng/5  $\mu$ l/hr) or 0.9% NaCl (5  $\mu$ l/hr) (panel A) followed by bolus of ANG II (5ng/2 $\mu$ l/30sec) (panel B). \* significant increase of MABP from baseline,  $p < 0.05$ ; + significant difference between treatments,  $p < 0.05$ .

angiotensin-aldosterone axis in the peripheral tissues (5, 6, 7). Specifically TNF- $\alpha$  activates AT1 receptors for ANGII in the cardiac fibroblasts (5).

The present study shows for the first time that administration of TNF- $\alpha$  into the ventricular system of the brain increases resting MABP and sensitises to the pressor effect of centrally administered AnGII in the rats with the post-infarct cardiac failure. In our previous study (8) *i.c.v.* administration of the same dose of TNF- $\alpha$  in the not infarcted rats did not evoke any appreciable increase in resting MABP although it did cause a weak augmentation of the ANGII-induced increase in blood pressure. The finding that the central pressor response to ANGII is significantly enhanced by TNF- $\alpha$  corresponds well to the data from the peripheral tissues showing multiple ways of co-operation between TNF- $\alpha$  and the renin-angiotensin system (5, 6, 7). One of the mechanisms which presumably may be involved in the TNF- $\alpha$  sensitising effect towards ANGII could be related to the increased availability of AT1 receptors in the neuronal membrane. With this regard, it has been shown that in the cardiac fibroblasts TNF- $\alpha$  increases synthesis of AT1 receptors (5).

In view of lack of changes in the resting MABP after *i.c.v.* administration of TNF- $\alpha$  in the not infarcted rats (8) the significant pressor effect of this procedure in the infarcted rats indicates significant augmentation of the hypertensive potentiality of the brain TNF- $\alpha$  system in the rats with the post-infarct heart failure. Elucidation of the mechanism of the greater central pressor effectiveness of TNF- $\alpha$  during the post-infarct state requires further studies. It may be caused by a greater expression of TNF- $\alpha$  receptors in the central nervous system or more effective activation by this peptide of the other pressor systems in the brain, for instance the renin-angiotensin or IL-1 $\beta$  systems. The later is also known for its sensitising effect towards the pressor action of ANGII (14). Beside, TNF- $\alpha$  was also found to modulate activity of the neurones by interacting with the ion channels (15). Therefore it is likely that in the infarcted rats it may directly influence excitability of the cardiovascular neurones involved in regulation of blood pressure to greater extent than in the not infarcted rats.

*Acknowledgements:* The study was supported by the Polish government grant nr 0085/B/P01/2008/35 and by the Medical University of Warsaw. Technical assistance of Marzana Tkaczyk and Marcin Kumosa is greatly appreciated.

Conflicts of interest statement: None declared.

#### REFERENCES

1. Oruss J, Roig E, Perez-Villa F *et al.* Prognostic value of serum cytokines in patients with congestive heart failure. *J Heart Lung Transplant* 2000; 19: 419-425.
2. Francis J, Chu Y, Johnson AK *et al.* Acute myocardial infarction induces hypothalamic cytokine synthesis. *Am J Physiol* 2004; 286: H2264-H2271.
3. Francis J, Zhang ZH, Weiss RM, Felder RB. Neural regulation of the proinflammatory cytokine response to acute myocardial infarction. *Am J Physiol* 2004; 287: H791-H797.

4. Guggilam A, Haque M, Kerut EK *et al.* TNF- $\alpha$  blockade decreases oxidative stress in the paraventricular nucleus and attenuates sympathoexcitation in heart failure rats. *Am J Physiol* 2007; 293: H599-H609.
5. Gurantz D, Cowling RT, Varki N *et al.* IL-1 $\beta$  and TNF- $\alpha$  upregulate angiotensin II type 1 (AT<sub>1</sub>) receptors on cardiac fibroblasts and are associated with increased AT<sub>1</sub> density in the post-MI heart. *J Mol Cell Cardiol* 2005; 38: 505-515.
6. Antonipillai I, Wang Y, Horton R. Tumor necrosis factor and interleukin-1 may regulate renin secretion. *Endocrinology* 1990; 126(1): 273-278.
7. Sasamura H, Nakazato Y, *et al.* Regulation of vascular type 1 angiotensin receptors by cytokines. *Hypertension* 1997; 30: 35-41.
8. Zera T, Ufnal M, Szczepanska-Sadowska E. Tumor necrosis factor- $\alpha$  sensitises to pressor action of centrally administered angiotensin II. *J Physiol Pharmacol* 2006; 57 (Suppl 2): 253.
9. Cudnoch-Jedrzejewska A, Szczepanska-Sadowska E, Dobruch J, *et al.* Fluid consumption, electrolyte excretion and heart remodeling in rats with myocardial infarct maintained on regular and high sodium intake. *J Physiol Pharmacol* 2005; 56: 599-610.
10. Francis J, Beltz T, Johnson AK, Felder RB. Mineralocorticoids act centrally to regulate blood-borne tumor necrosis factor- $\alpha$  in normal rats. *Am J Physiol* 2003; 285: R1402-R1409.
11. Kang YM, Zhang ZH, Johnson RF, *et al.* Novel effect of mineralocorticoid receptor antagonism to reduce proinflammatory cytokines and hypothalamic activation in rats with ischemia-induced heart failure. *Circ Res* 2006; 99(7): 758-66.
12. Zhang Z, Wei S, *et al.* Cardiovascular and renal sympathetic activation by blood-borne TNF- $\alpha$  in rat: the role of central prostaglandins. *Am J Physiol* 2003; 284: R916-R927.
13. Johnson AK, Grippo AJ. Sadness and broken hearts: neurohumoral mechanisms and comorbidity of ischemic heart disease and psychological depression. *J Physiol Pharmacol* 2006; 57(suppl.11): 5-29.
14. Ufnal M, Dudek M, Zera T, Szczepanska-Sadowska E. Centrally administered interleukin-1 $\beta$  sensitizes to the central pressor action of angiotensin II. *Brain Res* 2006; 1100: 64-72.
15. Viviani B, Gardoni F, Marinovich M. Cytokines and neuronal ion channels in health and disease. *Int Rev Neurobiol* 2007; 82: 247-63.

Received: October 24, 2008

Accepted: November 28, 2008

Author's address: Tymoteusz Zera, Department of Experimental and Clinical Physiology, Medical University of Warsaw, 00-927 Warsaw, 26/28 Krakowskie Przedmiescie Str, Poland. Tel/fax: + 4822 826 4586; e-mail: tyzer@amwaw.edu.pl