The influence of tumour necrosis factor-α on the cardiovascular system of anaesthetized rats

Abstract The effects of two vasoactive agents (adenosine A2A agonist, CGS 21680, and adrenoceptor agonist, noradrenaline) were examined on cardiac output (CO), heart rate (HR), blood pressure (BP), mean circulatory filling pressure (Pmcf), resistance to venous return, arterial resistance, dP/dt, plasma levels of NO2–/NO3–, and inducible nitric oxide synthase (iNOS) activity in lungs ex vivo, following treatment with tumour necrosis factor-α (TNF-α; 30 µg/kg) in anaesthetized rats. Treatment with TNF-α produced significant reduction in CO (41±2%), dP/dt (26±3%), BP (26±2%) and Pmcf (27±4%; n=6; mean±SEM), but increased arterial resistance. There were no significant changes in the plasma levels of NO2–/NO3– levels over time following treatment with TNF-α, but there was a significant increase (approximately twofold) in the activity of the iNOS in lungs of animals treated with TNF-α. Administration of CGS 21680 (1.0 µg/kg per min) significantly increased CO (44±6%), HR (12±2%), Pmcf (24±4%) and dP/dt (24±5%) in TNF-α-treated rats. CGS 21680 also significantly reduced arterial resistance (33±2%) without altering resistance to venous return in TNF-α-treated rats. While noradrenaline (1.0 µg/kg per min) infusion did not significantly increase CO, it did significantly increase HR (12±1%), BP (55±9%), Pmcf (47±5%), dP/dt (65±7%), resistance to venous return (64±20%), and arterial resistance (41±16%) in TNF-α-treated animals. The reduction in BP due to administration of TNF-α is the result of significant reduction in CO. Consequently, the decline in CO can be attributed to a combination of a negative inotropic effect as well as a reduction in Pmcf. It is evident that infusion with CGS 21680 could reverse the negative impact of TNF-α on CO by increasing dP/dt, Pmcf and HR as well as a reduction in arterial resistance. The fact that noradrenaline did not significantly increase CO in TNF-α-treated rats can be attributed to increased arterial resistance as well increase in resistance to venous return.

Keywords Arterial resistance · Cardiac output · Blood pressure · Mean circulatory filling pressure · Nitric oxide synthase · Resistance to venous return · Tumour necrosis factor-α

Introduction

Tumour necrosis factor-α (TNF-α) is a macrophage-derived polypeptide (MW 17KD) that exists as a trimer consisting of three identical polypeptide chains (for review see Grunfeld and Palladino 1990). It has been recognized for some time that TNF-α plays a key role in the initiation and regulation of the immune and inflammatory responses, and it has the ability to mediate some of the metabolic and cardiovascular responses during infection (Grunfeld and Palladino 1990). Earlier studies had indicated that systemic administration of TNF-α to animals could produce a syndrome similar to septic shock (Tracey et al. 1986; Kettelhut et al. 1987; Schirmer et al. 1989). Moreover, circulating levels of TNF-α have been noted to be elevated during septic shock under experimental and clinical situations (Tracey et al. 1987; Mitchie et al. 1988; Kengatharan et al. 1996). The cardiovascular sequelae in septic shock are, in part, due to the release of cytokines such as TNF-α and interleukins (Ruetten and Thiemermann 1997; Gardiner et al. 1998; Kraut et al. 1999). Furthermore, a link has been made between the rise in plasma concentration of the cytokine, TNF-α, and induction of nitric oxide synthase (NOS; Thiemermann et al. 1993; Kengatharan et al. 1996; Ruetten and Thiemermann 1997; Avontuur et al. 1998). Thus, it seems that the loss of vascular tone, reduction in cardiac output and hypotension in the state of circulatory shock has been linked to an over-production of nitric oxide (NO) following the induction of NOS (Thiemermann et al. 1993; Ayuse et al. 1995; Groeneveld et al. 1999).
However, there is also evidence in the literature which seems to suggest that TNF-α might be capable of producing its negative impact on the cardiovascular system independent of the process(es) that link it to induction of NOS. For example, it would seem that hypotension results within 50 min after the administration of TNF-α (Tracey et al. 1986), and this is contrary to the time-lines that have been described for the induction of NOS (Thiemermann 1994; Kengatharan et al. 1996). In addition, the initial reduction in cardiac output correlates quite well with a rise in the circulating levels of TNF-α (rather than induction of NOS) in experimental model of endotoxaemia (Forfia et al. 1998). Moreover, it is quite evident that the inhibition of NOS in endotoxaemia does not improve cardiac output, and in fact, it results in a further decline in cardiac output (Cheng and Pang 1998). Interestingly, TNF-α is not required for lipopolysaccharide (LPS)-mediated induction of NOS in rats (Xie et al. 1997). Taken together, it would be plausible to suggest that TNF-α could have a negative impact on the cardiac as well as the vascular system in vivo which is independent of an over-production of NO.

The administration of TNF-α produces a reduction in cardiac output and blood pressure, and associated with this is an increase in systemic vascular resistance (i.e. afterload; Mitaka et al. 1994). However, the influence of TNF-α on preload remains unknown. We have previously reported that administration of selective adenosine A2 receptor agonist, CGS 21680, in animals with acute or chronic heart failure resulted in improved cardiac output due to reduction in preload and afterload (Nekooeian and Tabrizchi 1998a, 1998b). In the present investigation our aim was to determine the impact of CGS 21680 and noradrenaline on cardiac output, heart rate, blood pressure, total body venous tone, resistance to venous return, arterial resistance, dP/dt, plasma levels of NO2-/NO3-, and NOS activity in lungs ex vivo, in animals treated with TNF-α.

Materials and methods

Surgical preparation of animals

Male Long-Evans rats (300–330 g) were anaesthetized with thiobutabarbital (100 mg/kg) i.p. Catheters (polyethylene tubing; I.D. 0.58 mm, O.D. 0.965 mm) were inserted into the left and right iliac arteries and veins. The left venous catheter was advanced into the inferior vena cava and used for the measurement of central venous pressure. The left arterial and right venous catheters were monitored continuously. Body temperature was maintained at 37°C using a heating lamp and monitored using a rectal thermometer. Arterial blood pressure and central venous pressures were recorded with a pressure transducer (Gould Statham, USA; Model PD23B) connected to an amplifier (DA 100A) which was connected to a universal interface module (UIM 100) which interfaced with an acquisition unit (MP 100). The data was collected using AcqKnowledge III (BIOPAC System, USA) and stored on an IBM-compatible microcomputer. Heart rate and dP/dt were calculated from the blood pressure and left ventricular pressure signal, respectively, using the AcqKnowledge III system. Cardiac output was measured using the reference sample method, and Pmcf was measured after circulation was transiently stopped by inflating the balloon in the right atrium. Final arterial pressure and venous plateau pressure were recorded at 5–7 s after the circulatory stop (Pang and Tabrizchi 1986).

All catheters were filled with heparinized saline (25 iu/ml). Body temperature was maintained at 37°C using a heating lamp and monitored using a rectal thermometer. Arterial blood pressure and central venous pressures were recorded with a pressure transducer (Gould Statham, USA; Model PD23B) connected to an amplifier (DA 100A) which was connected to a universal interface module (UIM 100) which interfaced with an acquisition unit (MP 100). The data was collected using AcqKnowledge III (BIOPAC System, USA) and stored on an IBM-compatible microcomputer. Heart rate and dP/dt were calculated from the blood pressure and left ventricular pressure signal, respectively, using the AcqKnowledge III system. Cardiac output was measured using the reference sample method, and Pmcf was measured after circulation was transiently stopped by inflating the balloon in the right atrium. Final arterial pressure and venous plateau pressure were recorded at 5–7 s after the circulatory stop (Pang and Tabrizchi 1986).

Measurement of cardiac output

This technique has been described in detail elsewhere (Pang 1983). Briefly, suspensions of microspheres (Mandel Canada; 15 µm diameter) labeled with 125I (20,000–22,000 in 150 µl) were infused into the left ventricle over a period of 10 s. The right arterial catheter was used to withdraw from the right femoral artery at the rate of 0.35 ml/min starting 15 s before microsphere injection using an infusion/withdrawal pump (Kd Scientific, USA; Model 120) for 1 min. The blood sample and syringes used for injection of microspheres or withdrawal of blood were counted for radioactivity at 80–160 keV using a dual channel automatic gamma counter (Clinic Gamma Counter, LKB Wallac, Canada; Model 1272). The withdrawn blood sample was slowly injected back into the animals immediately after counting of radioactivity.

Experimental protocol

Series I. Animals were randomly assigned to two groups (n=6) and they either received vehicle (twice distilled water) or TNF-α. After the completion of surgery, blood pressure, left ventricular pressure and heart rate were continuously monitored for 60 min at which time a blood sample (100 µl into heparinized tube taken from femoral artery) was taken for plasma measurements of NO2-/NO3- as well as haematoctrit measurements were made prior to the administration of vehicle or TNF-α. In addition, cardiac output and Pmcf were measured before the administration of vehicle or TNF-α. Subsequently each animal received either vehicle (0.6 ml/kg) or TNF-α (30 µg/kg). Following treatment of each animal with vehicle or TNF-α, five more blood samples were collected at 30 min, 1, 2, 3 and 4 h post-treatment for plasma NO2-/NO3- measurements. Furthermore, at 1, 2, 3 and 4 h post-treatment cardiac output and Pmcf measurements were made. As well, a final blood sample was taken for haematoctrit measurement at 4 h post-treatment with vehicle or TNF-α. After the completion of each experiment, the lungs were quickly excised, placed in liquid nitrogen and stored at −80°C.

Series II. Animals were randomly assigned to four groups (n=6). Two groups received either vehicle or TNF-α and were subsequently infused with CGS 21680. The other two groups also received vehicle or TNF-α but were subsequently infused with noradrenaline. Essentially, after the completion of surgery, blood pressure, left ventricular pressure and heart rate were continuously monitored for 60 min. At this time, a blood sample (100 µl into heparinized tube) was taken for plasma measurements of NO2-/NO3- as well a haematoctrit measurement was made, and cardiac output and Pmcf were measured prior to the administration of vehicle or TNF-α. Then each animal was treated with either vehicle (0.6 ml/kg) or TNF-α (30 µg/kg). Following treatment of each animal with vehicle or TNF-α, two more blood samples were collected at 1 h and 4 h post-treatment for plasma NO2-/NO3- measurements. In addition, at 1 h and 4 h post-treatment with vehicle or TNF-α, a cardiac output and Pmcf measurement was made. A final blood sample for