British Journal of Pharmacology (1998) 124, 493–498
http://www.stockton-press.co.uk/bjp

Effects of tumour necrosis factor-α on the coronary circulation of the rat isolated perfused heart: a potential role for thromboxane A_2 and sphingosine

N.J. Edmunds & [1]B. Woodward

Department of Pharmacology, University of Bath, Claverton Down, Bath BA2 7AY

1 The actions of tumour necrosis factor-α (TNF-α) on the coronary circulation were investigated in the rat isolated heart, perfused under constant flow, recirculating conditions.

2 An early increase in coronary perfusion pressure (CPP) was observed upon treatment with TNF-α (increase in CPP 10 min after TNF-α treatment: 45±12 mmHg *vs* control: 15±4 mmHg, $P<0.05$). The role of sphingosine, prostanoids and endothelins, in this coronary constrictor action, was investigated with the use of pharmacological inhibitors and antagonists.

3 The TNF-α induced increase in coronary tone was blocked by indomethacin, 10 μM (increase in CPP after 10 min: 13±4 mmHg *vs* TNF-α alone, $P<0.05$).

4 The thromboxane receptor antagonist GR32191, 10 μM, attenuated the TNF-α induced coronary constriction (12±2 mmHg *vs* TNF-α alone, $P<0.05$), as did the joint thromboxane A_2 synthesis inhibitor and receptor antagonist ZD1542, 10 μM (8±1 mmHg *vs* TNF-α alone, $P<0.05$).

5 The ceramidase inhibitor N-oleoylethanolamine (NOE), 1 μM, also blocked the TNF-α induced response (8±4 mmHg *vs* TNF-α alone, $P<0.05$).

6 In contrast, the coronary constrictor action of TNF-α was not inhibited by the endothelin$_{A/B}$ receptor antagonist bosentan, 3 μM (38±9 mmHg *vs* TNF-α, P=NS).

7 These data indicated that the early coronary vasoconstriction induced by TNF-α was mediated by both thromboxane A_2 and sphingosine, suggesting an interaction between both the sphingomyelinase and phospholipase A_2 metabolic pathways.

Keywords: TNF-α; sphingosine; thromboxane A_2; coronary circulation; vasoconstriction; GR32191; ZD1542; N-oleoylethanolamine; indomethacin; endothelin

Introduction

Tumour necrosis factor-α (TNF-α) is an important mediator of the profound circulatory changes observed during endotoxic shock (Tracey *et al*., 1986). TNF-α can mimic shock-like states, and TNF-α antibodies can protect against bacterial sepsis (Beutler *et al*., 1985; Tracey *et al*., 1987; Mathison *et al*., 1987). However, the precise role which this pro-inflammatory cytokine plays in shock has yet be fully elucidated. Metabolites of arachidonic acid (AA) could also play a role in the cardiovascular alterations observed during septic shock. Indeed, it has been postulated that the release of thromboxane A_2, a potent constrictor of smooth muscle cells, may in part compensate for the profound vasodilatation observed during shocked states (Cirino *et al*., 1996). But conversely, inhibitors of AA metabolism and thromboxane synthesis can be protective in shock (Fletcher & Ramwell 1977; Butler *et al*., 1983; Bult *et al*., 1985; Boughton-Smith *et al*., 1989; Mozes *et al*., 1991). Studies from another group have shown that TNF-α and other cytokines can cause *ex vivo* constriction of coronary vessels which is mediated by the release of endothelin-1 (ET-1) (Klemm *et al*., 1995a, b). Studies from the same laboratory have suggested that TNF-α mediated endothelin-1 (ET-1) release could be responsible for a coronary vasoconstriction seen *ex vivo* in shocked animals (Hohlfeld *et al*., 1995; Kengatharan *et al*., 1995).

TNF-α interacts with two different membrane bound receptors, p55 and p75, to assert its intracellular actions via a variety of different signalling pathways (for review see: Vilek & Lee, 1991; Camussi *et al*., 1991). TNF-α can activate phospholipase A_2 (PLA_2) (Clark *et al*., 1988), so having the potential to release AA, and hence increase the synthesis of prostaglandins (Elias *et al*., 1987) and thromboxane (Godfrey *et al*., 1987; Atkinson *et al*., 1990). In addition the sphingomyelinase (SMase) pathway is rapidly being recognized as another of the major signalling pathways exploited by TNF-α (Schutze *et al*., 1992; Dressler *et al*., 1992; Yang *et al*., 1993). A very recent study has implicated this pathway in the TNF-α-induced depression of cardiac myocyte contractility (Oral *et al*., 1997). When activated, sphingomyelinase causes the breakdown of the phospholipid, sphingomyelin to its metabolites ceramide and spingosine, both of which have potential second messenger functions (for review see: Kolesnick 1991; Hannun, 1994; Merrill *et al*., 1997). Interestingly, there appear to be strong interactions between PLA_2 activation and the SMase pathway, where released AA can stimulate SMase, and hence sphingomyelin breakdown (Jayadev *et al*., 1994). In addition to this, sphingosine can act synergistically with TNF-α, and other cytokines, to stimulate PLA_2 and cyclo-oxygenase enzymes (Candela *et al*., 1991; Ballou *et al*., 1992). Ceramide also has the ability to amplify the signal generated through PLA_2 activation (Hayakawa *et al*., 1993). A pharmacological tool which is incresingly being used to probe this pathway is N-oleoylethanolamine (NOE). NOE is an inhibitor of ceramidase (Sugita *et al*., 1975; Ramachandran *et al*., 1992), the enzyme in the SMase pathway which converts ceramide to sphingosine. This effect of NOE appears to be specific for the sphingolipid-mediated signalling pathway (Coroneos *et al*., 1995), and so can be used to

[1] Author for correspondence.

investigate the role of sphingosine upon activation of the sphingomyelinase pathway.

Whilst examining some of the metabolic actions of TNF-α in the rat isolated perfused heart (Edmunds & Woodward, 1997), we observed an early increase in coronary perfusion pressure, which is indicative of vasoconstriction in the coronary vessels. Clearly decreased substrate supply to the heart, which would occur during coronary vasoconstriction, could have serious consequences in shock conditions, where blood pressure is low and vital organ perfusion is already marginal. In this paper we describe our attempts to characterize the mechanism of the coronary vasoconstriction using NOE, indomethacin, inhibitors of thromboxane A_2 synthesis and receptors, and the $ET_{A/B}$ receptor antagonist bosentan. We present evidence to suggest a potential mechanism involving the interaction between sphingosine, as a metabolite of the sphingomyelinase pathway, and the vasoconstrictor, thromboxane A_2.

Methods

Throughout this study male Wistar rats, 280–310 g have been used. Animals were anaesthetized by an interperitoneal injection of sodium pentobarbitone (200 mg kg^{-1}) and then killed by cervical dislocation. The heart of each animal was isolated and perfused, via the aorta, according to the Langendorff technique, with prefiltered (Whatman No. 2), oxygenated (O_2 95%, CO_2 5%) Krebs-Henseleit solution of the following composition (mM): NaCl 118, $NaHCO_3$ 25, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ $7H_2O$ 1.2, $CaCl_2$ $6H_2O$ 1.2 and D-glucose 11.6; pH 7.4. The hearts were perfused with a constant flow of 10 ml min^{-1} and a temperature of 37°C. Coronary perfusion pressure (CPP) was recorded by a pressure transducer, and used as an index of coronary tone. A fluid filled clingfilm balloon was placed in the left ventricle, pressure changes were used as a measure of cardiac contractility. Left ventricular end diastolic pressure was set between 5 and 10 mmHg. Hearts were allowed to beat spontaneously throughout the experimental procedure. All hearts were perfused in a non-recirculating system for 30 min, before being switched to a recirculating system, with a total volume of 50 ml, for the remainder of the experiment, which lasted 90 min.

Recombinant human TNF-α (rhTNF-α), 20 ng ml^{-1}, was added to the perfusate at 25 min, 5 min before recirculation. In preliminary experiments a range of concentrations, up to 100 ng ml^{-1}, were investigated, and 20 ng ml^{-1} found to be optimum (results not shown), this was also a similar concentration to that used in other *in vitro* studies with TNF-α (Schulz *et al*., 1995). All antagonists used were added directly to the Krebs buffer after 5 min, to allow 20 min for equilibration before TNF-α addition. The cyclo-oxygenase inhibitor, indomethacin, 10 μM, was added to block prostanoid accumulation from TNF-α induced AA release. The thromboxane receptor antagonist GR32191, 10 μM (Lumley *et al*., 1988) and the joint thromboxane synthesis inhibitor and receptor antagonist, ZD1542, 10 μM (Brownlie *et al*., 1993), were both used to disrupt any thromboxane A_2 involvement in the actions of TNF-α. The ceramidase inhibitor NOE, 1 μM, was used to block sphingosine production from the SMase pathway (Oral *et al*., 1997). Ceramidase is the enzyme which converts ceramide to sphingosine. Bosentan, 3 μM, a non-specific endothelin receptor antagonist (Clozel *et al*., 1994) was used to block any potential actions of endogenous endothelin. Control experiments were conducted with all of the above agents in the absence of TNF-α.

Statistics

All results are expressed as mean ± s.e.mean. Statistical analysis was carried out, where appropriate, by one-way ANOVA coupled to either Dunett's test or Tukey's multiple comparison test. For non-parametric data a Mann-Whitney U test was performed. Differences were considered significant when $P<0.05$. Analysis was completed by the use of a computer programme (Minitab 10 for Windows).

Materials

Indomethacin and N-oleoylethanolamine were purchased from Sigma Chemical Co. (Poole, Dorset, U.K.). ZD 1542 (4(z)-6-[2S,4S,5R]-2-[1-methyl-1-(2-nitro-4-tolyloxy)ethyl]-4-(3-pyridyl)-1,3-dioxan-5-yl]hex-4-enoic acid) was a kind gift from Dr M.J. Wayne (Zenica). GR32191 (([1R-[α(Z),2β,3β,5α]]-(+)-7-[5-([1,1′-biphenyl]-4-ylmethoxy)-3-hydroxy-2-(1-piperidinyl)-cyclopentyl]-4 heptonoic acid) was a gift from Glaxo Pharmaceuticals. Bosentan was also a kind gift from Dr A.G. Roach (Rhone-Poulenc Rorer, Dagenham). rhTNF-α was a kind gift from Bayer (Slough).

Results

Effects of TNF-α in the rat isolated perfused heart

After switching perfusion from an open circuit to a recirculating system, CPP rose steadily throughout the 90 min experiment (Figure 2). Addition of TNF-α (20 ng ml^{-1}) to the Krebs buffer resulted in a rapid, and sustained rise in CPP, indicating constriction of the coronary vessels (Figures 1 and 3). It can be seen from the experimental trace in Figure 3 that the majority of this rise in CPP occurred within the first 10 min of TNF-α administration. CPP increase after 10 min was 45 ± 12 mmHg with TNF-α and 15 ± 4 mmHg in control hearts, $P<0.05$ (Figure 2). After this time CPP continued to rise in parallel with the control hearts. Interestingly, TNF-α caused an early and significant depression in left ventricular developed pressure (LVDP), which remained throughout the experiment (LVDP after 90 min: control *vs* TNF-α;

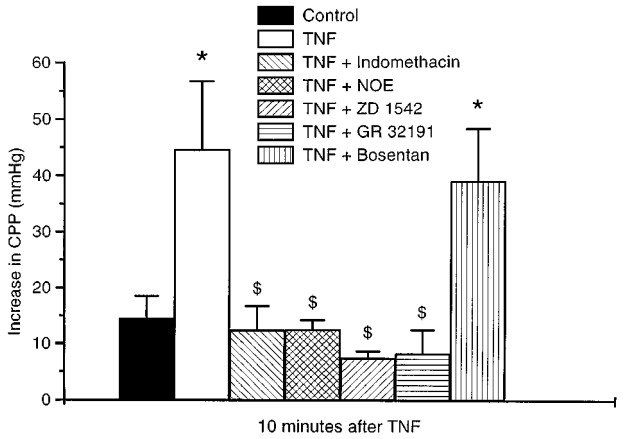

Figure 1 Effect of the antagonists indomethacin, 10 μM ($n=6$); NOE, 1 μM ($n=9$); ZD1542, 10 μM ($n=7$); GR32191, 10 μM ($n=7$); and bosentan, 3 μM ($n=7$) on the TNF-α 20 ng ml^{-1} ($n=10$) induced coronary vasoconstriction occurring 10 min after addition of TNF-α. Control in the absence of TNF-α $n=13$. *$P<0.05$ when compared with control data. $$P<0.05$ when compared with TNF-α data.

110±4 mmHg *vs* 82±10 mmHg, $P<0.05$), but did not alter heart rate (heart rate after 90 min: control *vs* TNF-α; 285±11 beats min^{-1} *vs* 288±12 beats min^{-1}, P=NS).

Effects of antagonists and inhibitors on the responses to TNF-α

NOE NOE (1 μM) completely blocked the TNF-α induced rise in CPP (Figure 1), without altering the tone of the coronary circulation in the absence of TNF-α (CPP after 90 min: control (n=13) *vs* NOE (n=6): 129±9 mmHg *vs* 113±16 mmHg, P=NS). NOE did not alter basal LVDP (LVDP before NOE *vs* after NOE: 110±4 mmHg *vs* 109±4 mmHg, P=NS) or heart rate (heart rate before NOE *vs* after NOE: 267±10 *vs* 263±9 beats min^{-1}, P=NS) throughout the experimental procedure.

Indomethacin TNF-α induced vasoconstriction was also completely inhibited by the cyclo-oxygenase inhibitor indomethacin (10 μM), which blocks the production of prostanoids from AA breakdown (Figure 1). In the absence of TNF-α indomethacin did not alter the increase in CPP over the 90 min perfusion period (Figure 2). Indomethacin also had no effect on basal parameters of LVDP (LVDP before indomethacin *vs* after indomethacin: 101±4 mmHg *vs* 105±4 mmHg, P=NS) or heart rate (heart rate before indomethacin *vs* after indomethacin: 263±4 *vs* 278±13 beats min^{-1}, P=NS).

Thromboxane A_2 antagonists In the light of the observation concerning indomethacin, it was decided to investigate the role of the vasoconstrictor prostanoid, thromboxane A_2, as a potential mediator of the vasoconstriction. To do this we used GR32191 (10 μM) and ZD1542 (10 μM). The TNF-α induced increase in CPP was completely inhibited by both of these drugs (Figure 1). Interestingly, unlike indomethacin, both GR32191 and ZD1542 caused a significant attenuation of the increase in CPP seen during the 90 min control perfusion (Figure 2). An unexpected action of GR32191, in both the presence and absence of TNF-α, was a large decrease in heart rate (heart rate before GR32191 *vs* after GR32191: 265±12 *vs* 205±16 beats min^{-1}, $P<0.05$) which could not be reversed by the muscarinic receptor antagonist, atropine (10 μM, data not shown). This was accompanied by a slight increase in basal LVDP (LVDP before GR32191 *vs* after GR32191: 96±2 mmHg *vs* 121±8 mmHg, $P<0.05$). ZD 1542 did not alter either LVDP (LVDP before ZD1542 *vs* after ZD1542: 98±7 mmHg *vs* 99±6 mmHg, P=NS) or heart rate (heart rate before ZD1542 *vs* after ZD1542: 272±8 *vs* 274±9 beats min^{-1}, P=NS).

Bosentan Addition of bosentan (3 μM) to the perfusate failed to alter the TNF-α-induced increase in CPP (Figure 2). This concentration of bosentan significantly inhibited the coronary constrictor actions of ET-1, 100 pM (increase in CPP after 5 min perfusion: ET-1: 97±6 mmHg *vs* bosentan and ET-1: 13±1 mmHg, $P<0.05$). Bosentan did not alter the basal CPP (CPP after 90 min in control hearts (n=13): 129±9 mmHg *vs* bosentan (n=5): 114±11 mmHg, P=NS), LVDP (LVDP before bosentan *vs* after bosentan: 104±2 mmHg *vs* 106±2 mmHg, P=NS) or heart rate (heart rate before bosentan *vs* after bosentan: 271±6 beats min^{-1}, P=NS).

Discussion

The aim of this study was to observe and characterize the acute actions of TNF-α on the coronary circulation of the rat isolated perfused heart under recirculating conditions. The major findings of these experiments can be summarized as follows: TNF-α, at a concentration relevant to serum levels observed during sepsis (Redl *et al*., 1993), caused an initial rise in CPP which was sustained throughout the experiment. This

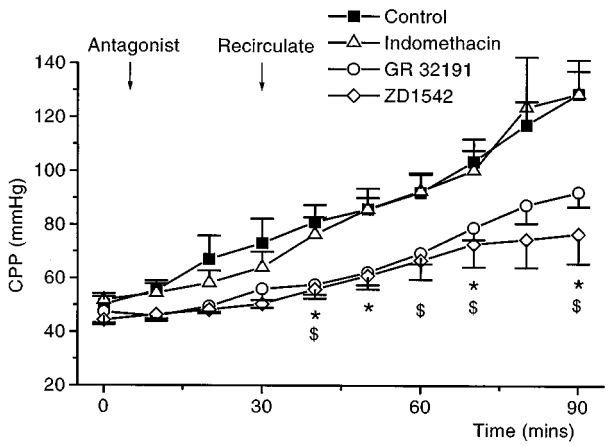

Figure 2 Graph showing the slow rise in coronary perfusion pressure (CPP) seen in control hearts (n=13), and the effects of GR32191, 10 μM (n=7); ZD1542, 10 μM (n=7); and indomethacin, 10 μM (n=6), on this parameter. *$P<0.05$ GR32191 *vs* control. $$P<0.05$ ZD1542 *vs* control.

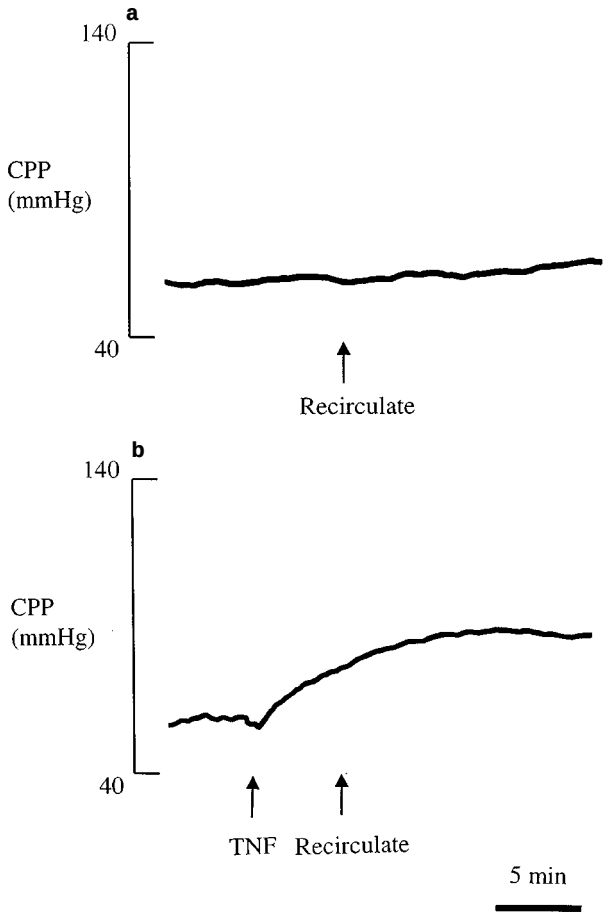

Figure 3 Typical experimental traces showing the coronary perfusion pressure (CPP) in (a) a control heart, and (b) after TNF-α 20 ng ml^{-1} administration. These traces are representative of 13 and 10 experiments, respectively.

increase in coronary tone appeared to be mediated by the vasoconstrictor prostanoid, thromboxane A_2, because it was completely abrogated by two different thromboxane antagonists. The fact that the ceramidase inhibitor NOE completely inhibited the TNF-α-induced coronary constriction suggests that sphingosine is also involved in this response. The $ET_{A/B}$ receptor antagonist bosentan did not inhibit this early action of TNF-α, indicating that the potent vasoconstrictor peptide, endothelin, is not involved.

Other studies have shown that TNF-α can activate PLA_2 (Clark *et al*., 1988; Hayakawa *et al*., 1993) and the SMase pathway (Kolesnick, 1991). However, we are unaware of any studies examining the effects of TNF-α on these pathways in the rat isolated perfused heart. Our studies showed a role for these pathways in the responses of a whole organ to TNF-α. The fact that the ceramidase inhibitor NOE (Sugita *et al*., 1975) and two thromboxane antagonists, GR32191 and ZD1542, inhibited the coronary constrictor actions of TNF-α raises a number of possibilities. Three potential explanations could account for the observations that we have made: firstly, TNF-α activates SMase to release sphingosine, which in turn causes the release of thromboxane A_2, to induce coronary vasoconstriction; secondly, TNF-α could activate the PLA_2 pathway, resulting in thromboxane A_2 synthesis, this may then release sphingosine to cause vasoconstriction. A third possibility is that two entirely different pathways are activated by TNF-α, which act in synergy to cause the constriction. It is not possible to tell from our studies which is the correct suggestion, although the third possibility is unlikely because the thromboxane antagonists and NOE both produced the same physiological response, complete inhibition of the TNF-α action, indicating that a sequential activation of the two pathways is more likely. Murohara *et al*. (1996) showed that sphingosine-induced coronary constriction in the pig was completely inhibited by indomethacin. Taken together with the results presented herein, this suggests that thromboxane A_2 is the final mediator of the TNF-α induced coronary constriction. Indeed, exogenous sphingosine has been shown to potentiate cytokine-induced prostaglandin production by increased activation of both PLA_2 and cyclo-oxygenase enzymes in fibroblasts (Candela *et al*., 1991; Ballou *et al*., 1992). In addition, AA has been shown to cause the stimulation of SMase and hence breakdown of sphingomyelin (Jayadev *et al*., 1994), and these authors postulated that TNF-α-induced activation of PLA_2 was essential for TNF-α instigated activation of SMase. This leads to the interesting possibility that a self potentiating mechanism exists in TNF-α signal transduction, whereby release of AA, by TNF-α activated PLA_2, could cause release of sphingosine, via activation of SMase, which could, in turn, increase AA breakdown as well as causing further activation of PLA_2. Thromboxane induced coronary vasoconstriction would explain why inhibitors of the cyclo-oxygenase enzyme and inhibitors of thromboxane itself can be protective in shocked states (Fletcher & Ramwell 1977; Beutler *et al*., 1983; Boughton-Smith *et al*., 1989; Mozes *et al*., 1991). Several recent studies have shown that following removal of hearts a short time, 15 to 30 min, after administration of lipopolysaccharide or TNF-α to the whole animal, there is a marked increase in the coronary tone in the rat isolated heart (Hohlfeld *et al*., 1995; Klemm *et al*., 1995a, b). This coronary vasoconstriction was associated with increased circulating endothelin levels and was blocked by the selective ET_A receptor antagonist, FR139317. The fact that in our experiments the dual ET receptor antagonist, bosentan, at a concentration that attenuates the intense coronary constrictor response to a 5 min infusion of 100 pM ET-1, did not affect the TNF-α induced rise in CPP, suggests that endothelins are not involved this early action of TNF-α. The reason for this apparent conflict is not at present clear. However, there are obvious differences in protocols followed. In the studies by the Vane group, the source of the proposed endothelins was not identified, and it is possible that overproduction of endothelins by organs other than the heart could have caused the large coronary vasoconstriction observed in these studies, clearly this would not apply to our studies where TNF-α was added directly to the perfusate of isolated heart.

A potential limitation of the work presented herein arises from a question over the specificity of the ceramidase inhibitor NOE, as it has been reported to inhibit cell swelling as well as calcium release from isolated mitochondria, leading to inhibition of calcium-dependant activation of PLA_2 (Epps *et al*., 1982; Broekemeier *et al*., 1985). The later of these observations could account for the inhibitory actions of NOE on thromboxane A_2 release. However, the concentrations of NOE required for the above effects were far higher (200 μM) than those used in our experiments (1 μM), and to our knowledge the concentration used in our experiments does not inhibit PLA_2. NOE has also been shown to inhibit specifically the activity of growth factors such as PDGF which utilize the sphingomyelinase pathway but not for those which employ other pathways, e.g. ET-1 (Coroneos *et al*., 1995). Also, a very recent study with cat ventricular myocytes has shown that NOE (1 μM) inhibits TNF-α-induced increases in sphingosine by 75% (Oral *et al*., 1997). Therefore we feel justified in using this concentration of NOE as a specific inhibitor of ceramidase.

Due to the structure of the coronary circulation within the whole heart, where coronary arteries penetrate deep into the ventricular walls, coronary resistance can be highly influenced by intramyocardial pressures (Katz, 1992). Therefore it is possible for changes in cardiac contractility to alter coronary tone, and so coronary perfusion pressure. Except for GR32191, none of the inhibitors or antagonists used adversely effected cardiac contractility. GR32191 caused a marked bradycardia which was accompanied by a slight increase in cardiac contractility. In spite of this, GR32191 affected the same physiological antagonism, of the TNF-α induced coronary vasoconstriction, as ZD1542. To this end we believe that the observations made herein are a result of the actions of the drugs used on the coronary circulation, and are independent of changes in cardiac contractility. The bradycardia seen with GR32191, was probably due to non specific actions of this drug, because the other thromboxane antagonist ZD1542 did not alter heart rate.

In the absence of TNF-α, we observed an increase in the basal coronary tone which was attenuated by both GR32191 and ZD1542, suggesting that in the rat isolated perfused heart there is a constant endogenous release of thromboxane A_2 which would accumulate under the recirculating conditions we have used. Pomposiello *et al*. (1997) have recently provided evidence to support this suggestion. However, indomethacin which would be expected to inhibit thromboxane production, did not alter CPP in the absence of TNF-α. This could be explained if there is a basal turnover of both vasodilator prostaglandins and constrictor thromboxane A_2, inhibiting the production of both of these might have no net effect of CPP. But blockade of thromboxane receptors and thromboxane synthesis could lead to an imbalance in the opposing forces, and hence vasodilatation due to vasodilator prostaglandins.

In summary, TNF-α caused an acute increase in coronary tone and evidence is presented to implicate sphingosine and thromboxane A_2 in this response. If this were to occur *in vivo*,

it would lead to a decrease in blood supply to the heart. This, together with a direct depressant action on cardiac contractility (Oral *et al*., 1997) would contribute to the overall depression of cardiac output seen septic shock.

This work has been funded by the British Heart Foundation, grant number: FS/95044.

References

ATKINSON, Y.H., MURRAY, A.W., KRILIS, S., VADAS, M.A. & LOPEZ, A.F. (1990). Human tumour necrosis factor-alpha (TNF-α) directly stimulates arachidonic acid release in human neutrophils. *Immunology,* **70,** 82–87.

BALLOU, L.R., CHAO, C.P., HOLNESS, M.A., BARKER, S.C. & RAGHOW, R. (1992). Interleukin-l-mediated PGE_2 production and sphingomyelin metabolism. *J. Biol. Chem.,* **28,** 20044–20050.

BEUTLER, B., MILSARK, J. & CERAMI, A. (1985). Passive immunisation against cachectin/tumour necrosis factor from lethal effect of endotoxin. *Science,* **229,** 869–871.

BOUGHTON-SMITH, N.K., HUTCHESON, I. & WHITTLE, B.J.R. (1989). Relationship between PAF-acether and thromboxane A_2 biosynthesis in endotoxin induced intestinal damage in the rat. *Prostaglandins,* **38,** 319–333.

BROEKEMEIER, K.M., SCHMID, P.C., SCHMID, H.H.O. & PFEIFFER, D.R. (1985). Effects of phospholipase A_2 inhibitors on ruthenium red-induced Ca^{2+} release from mitochondria. *J. Biol. Chem.,* **260,** 105–113.

BROWNLIE, R.P., BROWNRIGG, N.J., BUTCHER, H.M., GARCIA, R., JESSUP, R., LEE, V.J., TUNSTALL, S. & WAYNE, M.G. (1993). ZD1542, a potent thromboxane A_2 synthase inhibitor and receptor antagonist *in vitro*. *Br. J. Pharmacol.,* **110,** 1600–1606.

BULT, H., HERMAN, A.G. & RAMPART, M. (1985). Modification of endotoxin-induced haemological changes in the rabbit by methylprednisolone, $F(ab')_2$ fragments and rosarinic acid. *Br. J. Pharmacol.,* **84,** 317–327.

BUTLER, R.R., WISE, W.C., HALUSHKA, P.V. & COOK, J.A. (1983). Gentamicin and indomethacin in the treatment of spetic shock: Effects on prostacyclin and thromboxane A_2 production. *J. Pharmacol. Exp. Ther.,* **225,** 94–101.

CAMUSSI, G., ALBANO, E., TETTA, C. & BUSSOLINO, F. (1991). The molecular action of tumor necrosis factor-α. *Eur. J. Biochem.,* **202,** 3–17.

CANDELA, M., BARKER, S.C. & BALLOU, L.R. (1991). Sphingosine synergistically stimulates tumor necrosis factor α-induced prostaglandin E_2 production in human fibroblasts. *J. Exp. Med.,* **174,** 1363–1369.

CIRINO, G., SORRENTINO, R., CICALA, C., SORRENTINO, L. & PINTO, A. (1996). Indomethacin and thromboxane A_2/prostaglandin H_2 antagonist SQ29,548 impair in vitro contractions of aortic rings of ex vivo treated lipopolysaccharide rats. *J. Lipid. Med.,* **13,** 177–187.

CLARK, M.A., CHEN, M., CROOKE, S.T. & BOMALASKI, J.S. (1988). Tumour necrosis factor (Cachectin) induces phospholipase A_2-activating protein in endothelial cells. *Biochem. J.,* **250,** 125–132.

CLOZEL, M., BREU, V., GRAY, G.A., KALINA, B., LOFFER, B.M., BURRI, K., CASSAL, J.M., HIRTH, G., MULLER, M., NEIDHART, W. & RAMUZ, H. (1994). Pharmacological characterisation of bosentan, a new potent orally active non-peptide endothelin receptor antagonist. *J. Pharmacol. Exp. Ther.,* **270,** 228–235.

CORONEOS, E., MARTINEZ, M., MCKENNA, S. & KESTER, M. (1995). Differential regulation of sphingomyelinase and ceramidase activities by growth factors and cytokines. *J. Biol. Chem.,* **270,** 23305–23309.

DRESSLER, K.A., MATHIAS, S. & KOLESNICK, R.N. (1992). Tumor necrosis factor-α activates the sphingomyelin signal transduction pathway in a cell free system. *Science,* **255,** 1715–1718.

EDMUNDS, N.J. & WOODWARD, B. (1997). Effects of TNFα on insulin action in the isolated rat heart. *J. Mol. Cell. Cardiol.,* **29,** Fr57.

ELIAS, J.A., GUSTILLO, K., BAEDER, W. & FREUNDLICH, B. (1987). Synergistic stimulation of fibroblast prostaglandin production by recombinant interleukin 1 and tumor necrosis factor. *J. Immunology,* **138,** 3812–3816.

EPPS, D.E., PALMER, J.W., SCHMID, H.H.O. & PFEIFFER, D.R. (1982). Inhibition of permeability-dependant Ca^{2+} release from mitochondria by N-acylethanolamines, a class of lipid synthesized in ischaemic heart tissue. *J. Biol. Chem.,* **257,** 1383–1391.

FLETCHER, J.R. & RAMWELL, P.W. (1977). Modification, by asprin and indomethacin, of the haemodynamic and prostaglandin releasing effects of *E. coli* endotoxin in the dog. *Br. J. Pharmacol.,* **61,** 175–181.

GODFREY, R.W., JOHNSON, W.J. & HOFFSTEINN, S.T. (1987). Recombinant tumor necrosis factor and interleukin-1 both stimulate human synovial cell arachidonic acid release and phospholipid metabolism. *Biochem. Biophys. Res. Commun.,* **142,** 235–241.

HANNUN, Y.A. (1994). The sphingomyelin cycle and the second messenger function of ceramide. *J. Biol. Chem.,* **269,** 3125–3128.

HAYAKAWA, M., ISHIDA, N., TAKEUCHI, K., SHIBAMOTO, S., HORI, T., OKU, N., ITO, F. & TSUJIMOTO, M. (1993). Arachidonic acid-selective cytosolic phospholipase A_2 is crucial in the cytotoxic action of tumor necrosis factor. *J. Biol. Chem.,* **268,** 11290–11295.

HOHLFELD, T., KLEMM, P., THIEMERMANN, C., WARNER, T.D., SCHROR, K. & VANE, J.R. (1995). The contribution of tumour necrosis factor-α and endothelin-1 to the increase in coronary resistance in hearts from rats treated with endotoxin. *Br. J. Pharmacol.,* **116,** 3309–3315.

JAYADEV, S., LINARDIC, C.M. & HANNUN, Y.A. (1994). Identification of arachidonic acid as a mediator of sphingomyelin hydrolysis in response to tumor necrosis factor α. *J. Biol. Chem.,* **269,** 5757–5763.

KATZ, A.M. (1992). Structure of the heart and cardiac muscle. In: *Physiology of the Heart*. pp. 1–36. ed. Katz, A.M. New York: Raven Press.

KENGATHARAN, M., BATTISTINI, B., WARNER, T.D., THIEMERMANN, C. & VANE, J.R. (1995). Endothelin-1 mediates the increase in ex vivo coronary vascular resistance induced by hemorrhagic shock in the anesthetized rat. *J. Cardiovasc. Res.,* **26,** S422–S424.

KLEMM, P., WARNER, T.D., HOHFELD, T., CORDER, R. & VANE, J.R. (1995a). Endothelin-1 mediates *ex vivo* coronary vasoconstriction caused by exogenous and endogenous cytokines. *Proc. Natl. Acad. Sci. U.S.A.,* **92,** 2691–2695.

KLEMM, P., WARNER, T.D., CORDER, W.R. & VANE, J.R. (1995b). Endothelin-1 mediated coronary vasoconstriction caused by exogenous and endogenous cytokines. *J. Cardiovasc. Pharmacol.,* **26,** S419–S421.

KOLESNICK, R.N. (1991). Sphingomyelin and derivatives as cellular signals. *Prog. Lipid Res.,* **30,** 1–38.

LUMLEY, P., SEAGER, K. & WHITE, B. (1988). The TP-receptor blocking drugs GR32191 and BM13.177 have a differential profile of action on vascular smooth muscle. *Br. J. Pharmacol.,* **95,** 685P.

MATHISON, J.C., WOLFSON, E. & ULEVITCH, R.J. (1987). Participation of tumor necrosis factor in the mediation of gram negative bacterial lipipolysaccharide-induced injury in rabbits. *J. Clin. Invest.,* **81,** 1925–1937.

MERRILL, A.H., SCHMELZ, E.-M., DILLEHAY, D.L., SPIEGEL, S., SHAYMAN, J.A., SCHROEDER, RILEY, R.T., VOSS, K.A. & WANG, E. (1997). Sphingolipids- the enigmatic lipid class: biochemistry, physiology and pathophysiology. *Toxicol. Appl. Pharmacol.,* **142,** 208–225.

MOZES, T., ZIJLSTRA, F.J., HEILIGERS, J.P.C., TAK, C.J.A.M., BENEFRAIM, S., BONTA, I.L. & SAXENA, P.R. (1991). Sequential release of tumour necrosis factor, platelet activating factor and eicosanoids during endotoxin shock in anaesthetized pigs; protective effects on indomethacin. *Br. J. Pharmacol.,* **104,** 691–699.

MUROHARA, T., KUGIYAMA, K., OHGUSHI, M., SUGIYAMA, S., OHTA, Y. & YASUE, H. (1996). Effects of sphingomyelinase and sphingosine on arterial vasomotor regulation. *J. Lipid. Res.,* **37,** 1601–1608.

ORAL, H., DORN, G.W. & MANN, D.L. (1997). Sphingosine mediates the immediate negative inotropic effects of tumor necrosis factor-α in the adult mammalian cardiac myocyte. *J. Biol. Chem.,* **272,** 4836 – 4842.

POMPOSIELLO, S., YANG, X.P., LIU, Y.H., SURAKANTI, M., RHALEB, N.E., SEVILLA, M. & CARRETERO, O.A. (1997). Autocoids mediate coronary vasoconstriction induced by nitric oxide synthesis inhibition. *J. Cardiovasc. Pharmacol.,* **30,** 599 – 606.

RAMACHANDRAN, C.K., MURRAY, D.K. & NELSON, D.H. (1992). Enzymatic hydrolysis of sphingomyelinase ion 3T3-L1 cells modulation by dexamethasone. *Biochem. Archives,* **8,** 369 – 377.

REDL, H., SCHLAG, G., BAHRAMI, S., DAVIES, J., WAAGE, A., CESKA, M., BUURMAN, W.A. & ADOLF, G. (1993). The cytokine network in trauma and sepsis I: TNF and IL-8. In *Pathophysiology of Shock, Sepsis and Organ Failure*. ed. Schlag, G. & Redl, H. pp. 468 – 490. Berlin Heidelberg: Springer-Verlag.

SCHULZ, R., PANAS, D.L., CATENA, R., MONCADA, S., OLLEY, P.M. & LOPASCHUK, G.D. (1995). The role of nitric oxide in cardiodepression induced by interleukin-1β and tumour necrosis factor-α. *Br. J. Pharmacol.,* **114,** 27 – 34.

SCHULTZE, S., POTTHOFF, K., MACHLEIDT, T., BERKOVIC, D., WIEGMANN, K. & KRONKE, M. (1992). TNF activates NF-κB by phosphatidylcholine-specific phospholipase C-induced 'acidic' sphingomyelin breakdown. *Cell,* **71,** 765 – 776.

SUGITA, M., WILLIAMS, M., DULANEY, J.T. & MOSER, H.W. (1975). Ceramidase and ceramide synthesis in human kidney and cerebellum. *Biochem. Biophys. Acta,* **398,** 125 – 131.

TRACEY, K.J., BEUTLER, B., LOWRY, S.F., MERRYWEATHER, J., WOLPE, S., MILSARK, I.W., HARIRI, R.J., FAHEY III, T.J., ZENTELLA, A., ALBERT, J.D., SHIRES, G.T. & CERAMI, A. (1986). Shock and tissue injury induced by recombinant human cachectin. *Science,* **234,** 470 – 474.

TRACEY, K.J., FONG, Y., HESSE, D.G., MANOGUE, K.R., LEE, A.T., KUO, G.C., LOWRY, S.F. & CERAMI, A. (1987). Anti-cachectin/TNF monoclonal antibodies prevent septic shock during lathal bacteraemia. *Nature,* **330,** 662 – 664.

VILCEK, J. & LEE, T.H. (1991). Tumor necrosis factor. New insights into the molecular mechanisms of its multiple actions. *J. Biol. Chem.,* **266,** 7317 – 7316.

YANG, Z., COSTANZO, M., GOLDE, D.W. & KOLESNICK, R.N. (1993). Tumor necrosis factor activation of the sphingomyelin pathway signals nuclear factor κB translocation in intact HL-60 cells. *J. Biol. Chem.,* **268,** 20520 – 20523.

(Received December 1, 1997
Revised February 26, 1998
Accepted March 2, 1998)