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The contribution of tumour necrosis factor-α and endothelin-1 to the increase of coronary resistance in hearts from rats treated with endotoxin

[2]Thomas Hohlfeld, [3]Peter Klemm, [1]Christoph Thiemermann, Timothy D. Warner, [2]Karsten Schrör & John R. Vane

The William Harvey Research Institute, St. Bartholomew's Hospital Medical College, Charterhouse Square, London EC1M 6BQ

1 Inflammatory disease states predispose to myocardial infarction. Here we have investigated the effects of a systemic inflammatory response syndrome, i.e. lipopolysaccharide (LPS)-induced circulatory shock in rats, on coronary vascular tone.

2 Anaesthetized rats were given LPS (10 mg kg^{-1}, i.v.) and the hearts excised 180 min later for isolated perfusion at constant flow by the Langendorff technique. Once the *ex vivo* perfusion was started, the perfusion pressure strongly increased in these hearts compared to hearts from control rats (130±3 vs. 49±3 mmHg after 10 min). This increase in coronary resistance was not associated with a reduction in endothelial cell function, for the vasodilator responses to bradykinin were unchanged.

3 When hearts were removed 30 min after the injection of LPS, the LPS-induced rise in perfusion pressure was delayed. No changes in perfusion pressure were seen when the hearts were removed 15 min after the injection of LPS. Pre-treatment with cycloheximide or an anti-tumour necrosis factor-α (TNF-α) antibody or continuous infusion *in vivo* and *in vitro* of the endothelin ET_A receptor selective antagonist FR 139317, greatly decreased the increase in coronary vascular resistance induced by LPS.

4 These data suggest that TNF-α may induce the release of endothelin-1 (ET-1) and that this mediates at least part of the coronary vasoconstriction. This hypothesis is supported by the demonstration that LPS administration increased the circulating levels of both TNF-α and ET-1.

5 We conclude, therefore, that in inflammatory disease states, such as LPS-induced septic shock, there is the sequential release of TNF-α and endothelin-1 which leads to an increase in coronary vascular tone and so a predisposition to myocardial ischaemia. Inactivation of TNF-α by an antibody as well as ET_A receptor blockade by a selective antagonist may effectively interfere with this pathway.

Keywords: Coronary blood flow; bacterial lipopolysaccharide; endothelin receptors; FR139317; tumour necrosis factor; cytokines

Introduction

Severe inflammatory disorders, such as septic shock, may cause marked alterations in the formation of vasoactive mediators. For instance, increases in the formation of the vasodilator mediators prostacyclin (Hardie & Olsson, 1987; Oettinger *et al.*, 1987) and nitric oxide (NO) (Thiemermann & Vane, 1990; Joulou-Schaeffer *et al.*, 1990, Kilbourn *et al.*, 1990a,b; Rees *et al.*, 1990) have been demonstrated. There is also evidence that the release of vasoconstrictor substances, such as noradrenaline (see Benedict & Grahame-Smith, 1978), endothelin-1 (Sugiura *et al.*, 1989; Nakamura *et al.*, 1991; Vemulapalli *et al.*, 1991) and neuropeptide Y (Pernow *et al.*, 1990) is increased. Thus, it may well be that shifts in the balance of production of these mediators account for the alterations of organ blood flow seen in septic shock. Such a shift may well be of particular importance in the coronary vasculature and in the development of myocardial dysfunction and injury (Han *et al.*, 1994).

This suggestion is supported by several lines of evidence. Firstly, patients with myocardial infarction have increased circulating lipopolysaccharide (LPS)-specific immune complexes (Leinonen *et al.*, 1990; Saikku 1993), suggesting that gram-negative infection may precipitate myocardial ischaemia (Nieminen *et al.*, 1993). Indeed, the experimental administration of endotoxin increases coronary microvascular resistance, even when it is applied at very low, non-lethal doses (McDonough *et al.*, 1986). Secondly, cytokines such as tumour necrosis factor (TNF)-α and interleukin-1β, which are produced in large amounts in septic shock, may cause cardiomyocyte injury (Hansen *et al.*, 1994) and myocardial infarction in patients without coronary artery disease (Nora *et al.*, 1989; Kragel *et al.*, 1990). Thirdly, cytokines including interleukin (IL)-2, TNF-α and interferon-γ also appear to contribute to coronary stenosis in patients with mucocutaneous syndrome (Kawasaki vasculitis), a cause of ischaemic heart disease and myocardial infarction in children. Thus, the release of these cytokines is of predictive value for the appearance of myocardial ischaemia in this disease, both experimentally (Tomita *et al.*, 1993) and clinically (Lin *et al.*, 1991; Leung, 1991).

Here, we have examined *ex vivo* the changes in coronary vascular resistance induced by LPS treatment *in vivo*. Selective inhibitors of the production and effects of TNF-α and endothelin-1 (ET-1) have been used to characterize the involvement of these mediators in the changes in coronary reactivity observed and to provide a basis for pharmacological prevention of cytokine-induced coronary vasoconstriction.

Methods

In vivo *preparation*

Male Wistar rats (250-300g, Glaxo, Greenford, Middx., U.K.) were anaesthetized with sodium thiopentone (120 mg kg^{-1}, i.p.). The trachea was cannulated to facilitate

[1]Author for correspondence.
[2]Present address: Institut für Pharmakologie, Universität Düsseldorf, Düsseldorf, Germany.
[3]Present address: Department of Pharmacology, CASSELLA AG, Frankfurt am Main, Germany.

respiration and a catheter was implanted into the right jugular vein for the injection of drugs. Body temperature of the anaesthetized animals was maintained at 37°C by a homeothermic blanket connected to a rectal probe (Biosciences, Sheerness, Kent, UK). All animals were then left for a period of 30 min to stabilize after which time they received a bolus i.v. injection of lipopolysaccharide (LPS, *E. coli* 127: BO8, 10 mg kg^{-1}) or vehicle (as control). After a further 15–180 min, the rats were killed (see below) and the hearts rapidly excised for *ex vivo* perfusion by the Langendorff method ($n=5-6$ per group).

In experiments examining the effects of dexamethasone (10 mg kg^{-1}), cycloheximide (3 mg kg^{-1}) or antibody against human recombinant TNF-α (TNF-α-Ab, 3 mg kg^{-1}), the compounds were given as a brief 3 min infusion, 30 min before the administration of LPS ($n=4-6$ per group). The hearts of these rats were removed for isolated perfusion 180 min after LPS was injected. To investigate the effects of endothelin receptor antagonism, the ET_A receptor antagonist FR 139317 (Sogabe *et al.*, 1993) was infused at a dose of 0.3 mg kg^{-1} min^{-1} ($n=6$) starting from 1 min before the injection of LPS and continuing until the animal was killed. FR 139317 (1 μM) was then included in the Krebs buffer in the subsequent *ex vivo* coronary perfusion. These doses of FR 139317 were found completely to block the pressor effect of ET-1 (0.5 nmol kg^{-1}) *in vivo* and the coronary vasoconstrictor effects of ET-1 (10 nM) *in vitro* (data not shown). Separate sets of hearts from LPS-treated rats received vehicle instead of the inhibitors, serving as control groups in these experiments.

To elucidate the effects of LPS on the plasma levels of TNF-α and ET-1, animals ($n=5-6$ per group) were prepared as above and blood (0.3–0.4 ml) removed from the carotid artery at 0, 15, 60, 90, 120, 150, 180 or 210 min, and collected into Eppendorff tubes containing heparin (30 u ml^{-1}, final concentration). The Eppendorff tubes were centrifuged (Heraeus, Biofuge 15) at 15,000 r.p.m. for 3 min and the plasma removed and stored at −80°C until assay.

Rat isolated heart perfusion

At the end of the *in vivo* experiment, the rats were heparinized (250 u, i.v.), the thorax rapidly opened and the hearts quickly excised and placed in cold (4°C) saline until contractions had ceased (approx. 5 s). Within 1 min the hearts were then tied to the steel cannula of a Langendorff perfusion apparatus, and perfused at a constant flow of 12 ml min^{-1} with warmed (37°C) and gassed (95% O_2, 5% CO_2) Krebs buffer. The perfusate consisted of (mM): NaCl 118, KCl 4.5, $NaHCO_3$ 25, NaH_2PO_4 1.4, $MgSO_4$ 1.2, $CaCl_2$ 1.4, glucose 11, pH 7.4 and was pre-filtered before use through a 1 μm filter disk (Millipore, Eschborn, Germany). The perfusion pressure in the aortic line was continuously recorded by an Elcomatic type 750 pressure transducer connected to a chart recorder (Grass model 79) as a measure of coronary resistance. A latex balloon, inserted into the left ventricle via the mitral valve, was linked to a transducer and chart recorder as above for measurement of left ventricular developed pressure. This balloon was inflated after insertion to reach an end-diastolic left ventricular pressure of 5 mmHg.

Preliminary experiments have shown that the perfussion pressures of hearts from LPS-treated rats given cycloheximide and dexamethasone did not change within 10 and 120 min of isolated perfusion. These hearts were perfused for 30 min. In contrast, hearts from rats receiving FR 139317 showed a time-dependent variation in coronary resistance during perfusion. Therefore, the perfusion pressures were recorded for 120 min in these experiments. The perfusion pressures of hearts from rats treated with LPS and TNF-α-Ab were tendentially higher than in the control experiments without LPS administration. In order to detect a potential LPS-induced increase of coronary resistance at later times of perfusion, these hearts were also perfused for 120 min.

In some experiments, to examine vasodilator responses to bradykinin, hearts from control animals were infused with ET-1 (10^{-8} M, for 3 min) to increase the basal perfusion pressure. Vasodilatations induced by bradykinin (3×10^{-10} to 10^{-7} M) in these hearts or in hearts from LPS-treated animals were then recorded.

Assay of TNF-α and ET-1 in plasma samples

The content of TNF-α in plasma samples (50 μl, $n=6$) was determined in 96-well plates (mouse TNF-α ELISA kit, Genzyme, MA, U.S.A.). Binding was detected by a peroxidase-conjugated polyclonal anti-mouse TNF-α antibody using tetramethylbenzidine as a substrate. Following acidification (sulphuric acid, 0.5 M final), the absorbance of each well was measured at 450 nm (Anthos Labtec Instruments, Uckfield, East Sussex, U.K.).

For the detection of ET-1, plasma samples (200 μl, $n=5$) were diluted with 800 μl saline, acidified with 250 μl HCl (2 M) and centrifuged (10,000 *g*, for 5 min) before being loaded onto Amprep 500 mg C_{18} columns pre-equilibrated with methanol (2 ml) and water (2 ml). After being washed with water (5 ml) and 0.1% trifluoroacetic acid (TFA), the columns were eluted with 80% methanol + 0.1% TFA (2 ml) and the eluent dried down under N_2. Samples were reconstituted and assayed by use of an ET-1 ELISA system (Biotrak, Amersham Life Science, Little Chalfont, U.K.). The assay plates (96-well) were read as above.

Histology

To quantify the extent of intravascular coagulation and the accumulation of inflammatory cells, the hearts of two control and two LPS (10 mg kg^{-1}, 180 min) treated rats were quickly excised, placed in formaldehyde (4%) and subjected to standard light microscopy (hematoxylin/eosin stain).

Drugs and solutions

LPS (serotype 127:BO8) was purchased from Sigma (Poole, U.K.) and was suspended in sterile 150 mM NaCl at a concentration of 10 mg ml^{-1}. Sodium thiopentone was obtained from May and Baker Ltd. (Dagenham, Essex, UK). FR 139317 ((**R**)-2[(**R**)-2-[(**S**)-2-1-(hexahydro-1H-azepinyl) carbonyl] amino-4-methylpentanoyl] amino-3[3-(1-methyl-1H-indonyl) proprionyl] amino-3-(2-pyridyl) propionic acid) (Sogabe *et al.*, 1993) was synthesized by Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company. FR 139317 was freshly dissolved before use as a stock solution of 4.06 mg ml^{-1} in 10% dimethylsulphoxide (DMSO) and was further diluted into saline or Krebs buffer before use so that the final DMSO concentration did not exceed 0.1%. TNF-α-Ab was a generous gift of Therapeutic Antibodies Inc. (Nashville, U.S.A.) and was dissolved in saline immediately before use. Endothelin-1 was purchased from Peptide Institute (Osaka, Japan). Bradykinin and cycloheximide were bought from Sigma. The Krebs buffer salts were from BDH (Lutterworth, UK).

Statistical evaluation

All results are expressed as mean values ± s.e.mean of *n* observations. Statistical analysis was performed by Student's *t* test for unpaired observations or one-way analysis of variance followed by Bonferroni's test, as appropriate. *P* values of less than 0.05 were considered to be significant.

Results

Treatment of rats with LPS (10 mg kg^{-1}) resulted within 180 min in a mortality of 7 out of 75 animals. Thus, overall mortality was 9%, and mortality was similar in all of the animal groups studied. Data from experiments in which the rats

died before the end of the *in vivo* period were excluded from data analysis.

Effect of LPS treatment on coronary perfusion pressure

The perfusion pressures of hearts taken from control animals were 33±3, 53±2 and 74±5 mmHg after 1, 20 and 120 min, respectively. In hearts taken from rats treated with LPS (10 mg kg^{-1}, 180 min), there was a marked coronary vasoconstriction such that the perfusion pressures after 1, 20 and 120 min were 65±12, 127±2 and 110±4 mmHg, respectively ($P<0.05$ from control for all) and the maximum perfusion pressure (130±3 mmHg) was reached after only 10 min perfusion *ex vivo* (Figure 1). When LPS was administered 15 min before the hearts were removed for *ex vivo* perfusion, there was no detectable coronary vasoconstriction (Figure 1), although there was when the hearts were removed 60 min after LPS administration. In these latter hearts, the perfusion pressure increased during the *in vitro* period, significantly exceeding the control after 5 min of perfusion and after 90 min not being different from those seen in hearts taken from rats treated with LPS for 180 min.

The increase in coronary perfusion pressure in hearts taken from rats treated with LPS for 180 min was not associated with oedema formation (dry/wet weight ratio: control hearts, 23.6±0.2%; LPS-treated hearts, 23.2±0.2%), nor an accumulation of inflammatory cells as judged by light microscopy (not shown). There were also no significant differences in left ventricular developed pressure between the hearts taken from control and LPS-treated rats (control, 75±5; LPS, 67±7 mmHg, after 20 min of isolated perfusion, $P>0.05$).

Endothelial function and cyclo-oxygenase metabolites

For the assessment of endothelial function, increasing concentrations of bradykinin (0.3–100 nM) were infused (2 min) into the vasculature of hearts taken from LPS-treated rats and in control hearts preconstricted by infusion of ET-1. This caused similar falls in perfusion pressure (Table 1), indicating that LPS-treatment did not compromise endothelial function. In addition, the coronary resistance in hearts from LPS-treated rats was not affected by administration of indomethacin (10 μM) (data not shown).

Modification of the effect of LPS by cycloheximide and dexamethasone

Pretreatment of rats with cycloheximide (3 mg kg^{-1}, i.v.) 30 min before LPS greatly reduced the increase of coronary resistance at all time points (Figure 2). Thus, the differences in coronary resistance between hearts removed from cycloheximide-pretreated, LPS-challenged rats and control animals were less than 10 mmHg throughout the time (30 min) of Langendorff perfusion. Dexamethasone (10 mg kg^{-1}, i.v.) given 30 min before LPS did not modify the effect of LPS (10 mg kg^{-1}, 180 min) on the coronary vascular resistance (Figure 2).

Plasma levels of TNF-α

LPS (10 mg kg^{-1}) injection was associated with a time-dependent increase in the plasma levels of TNF-α that reached a peak value of 3.8±0.2 ng ml^{-1} after 90 min before declining to less than 10% of the peak value after 150 min (Figure 3). This rise was almost completely suppressed by pretreatment with cycloheximide (290±65 pg ml^{-1} 90 min after LPS, $P<0.05$ vs LPS alone), but unaffected by dexamethasone.

Neutralisation of TNF-α by a specific antibody

Administration of TNF-α-Ab 30 min before LPS markedly attenuated the LPS-induced rise in coronary resistance during

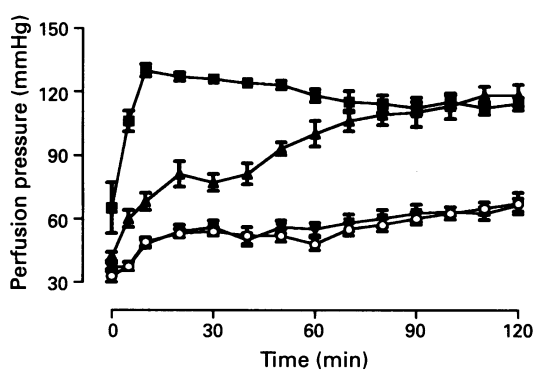

Figure 1 Perfusion pressures of hearts removed from control rats (○, $n=6$), and from rats treated *in vivo* with lipopolysaccharide (LPS, 10 mg kg^{-1}, i.v.) 15 min (▼, $n=5$), 60 min (▲, $n=6$) and 180 min (■, $n=6$) before hearts were taken. Each point with a vertical bar represents the mean±s.e.mean of n determinations.

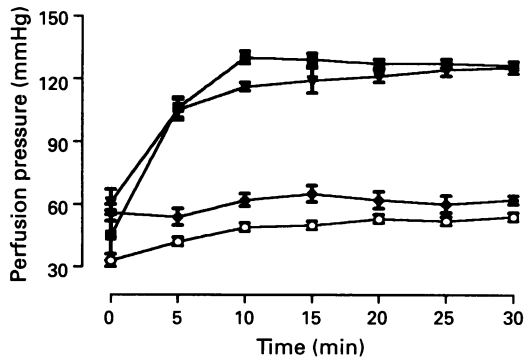

Figure 2 Changes in perfusion pressure of isolated hearts taken from control rats (○, $n=6$) and from those treated *in vivo* with lipopolysaccharide (LPS, 10 mg kg^{-1}, i.v.) in the presence of vehicle (■, $n=6$), dexamethasone (▼, 10 mg kg^{-1}, i.v., $n=6$) or cycloheximide (◆, 3 mg kg^{-1}, i.v., $n=6$). Each point with a vertical bar represents the mean±s.e.mean of n determinations.

Table 1 Bradykinin-induced coronary relaxation in isolated perfused hearts, either obtained from rats pretreated with lipopolysaccharide (LPS, 10 mg kg^{-1}, for 180 min) or in control hearts precontracted with endothelin-1 (ET-1, 10 nM)

Bradykinin [μM]	*0*	*0.3*	*1*	*3*	*10*	*30*	*100*
LPS ($n=5$)	125±3	122±2	116±3	109±4	88±7	71±8	66±5
ET-1 ($n=4$)	126±6	126±5	124±4	118±5	103±7	83±5	61±4

In both groups, bradykinin was applied into the aortic cannula as a 2 min short-term infusion. The values are mmHg perfusion pressure at a perfusion rate of 12 ml min^{-1}.
Each value represents the mean±s.e.mean of n separate experiments.

the *ex vivo* perfusion period (Figure 4) such that the perfusion pressure was no longer significantly different from that in control hearts.

Plasma levels of endothelin-1

The plasma levels of immunoreactive ET-1 in control rats did not exceed 20 pM (Figure 5). However, in the plasma from LPS-treated animals there was a significant increase in the levels of ET-1 between 60 and 180 min with the maximum concentration of ET-1, 186±39 pM (control 20±7 pM, $P<0.05$ vs LPS), being reached at 120 min.

ET_A receptor blockade

FR 139317 infused at a dose of 0.3 mg kg^{-1} min^{-1} blocked the rise in mean arterial blood pressure evoked by i.v. administration of 1 nmol ET-1 and at a concentration of 1 μM prevented the increase in coronary resistance elicited by 10 nM ET-1 in the isolated perfused heart (not shown).

The perfusion pressures of hearts removed from rats treated with LPS (180 min) and exposed to FR 139317 (0.3 mg kg^{-1} min^{-1} *in vivo* plus 1 μM *in vitro*) were significantly lower than those of hearts taken from rats exposed to LPS alone (Figure 6). However, coronary resistance was only reduced to the values of control hearts after 120 min of perfusion *ex vivo*.

Discussion

Here we show that exposure to LPS *in vivo* causes a time-dependent, sustained increase in coronary perfusion pressure *ex vivo* associated with an elevation in the arterial level of ET-1. This coronary vasoconstriction is significantly prevented by administration of TNF-α-Ab or the endothelin ET_A receptor-selective antagonist FR 139317 (Sogabe *et al.*, 1993). Together these data indicate that the increase in coronary perfusion pressure induced by LPS is due, at least in part, to a pronounced release of TNF-α which enhances the endogenous production and/or release of ET-1 and so promotes coronary vasoconstriction.

This conclusion is supported by several lines of evidence. Firstly, the increase in coronary perfusion pressure induced by LPS treatment *in vivo* was a time-dependent phenomenon. Thus, hearts removed from rats 15 min after LPS injection had perfusion pressures that were not different from those in hearts removed from control animals, whereas a slowly developing

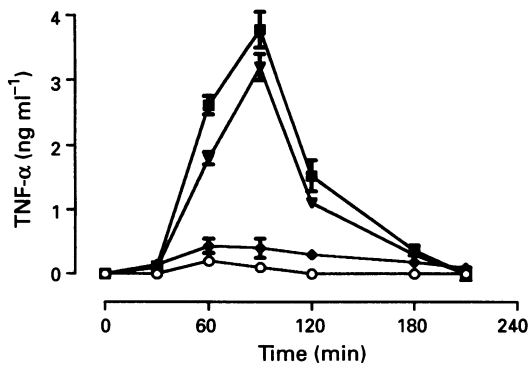

Figure 3 Changes in circulating levels of tumour necrosis factor-α (TNF-α) in control rats (○, $n=6$) and in animals treated with lipopolysacchride (LPS, 10 mg kg^{-1}, i.v.) in the presence of vehicle (■) dexamethasone (▼, 10 mg kg^{-1} min^{-1} i.v., $n=6$) or cycloheximide (◆, 3 mg kg^{-1}, i.v., $n=6$). Each point with a vertical bar represents the mean±s.e.mean of n determinations.

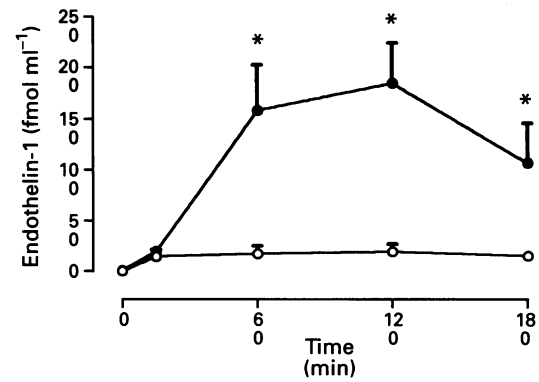

Figure 5 Changes in circulating levels of endothelin-1 (ET-1) in rats treated with lipopolysaccharide (LPS, ●, 10 mg kg^{-1}, i.v., $n=5$) or vehicle (○, $n=6$). Each point with a vertical bar represents the mean±s.e.mean of n determinations.

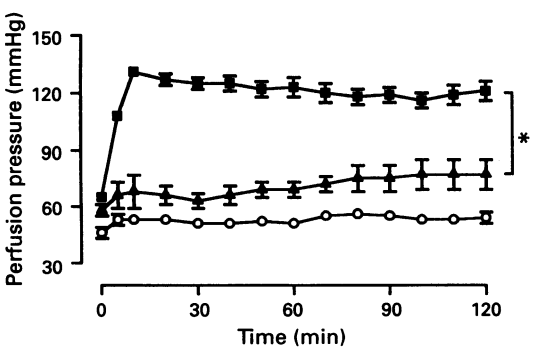

Figure 4 Changes in perfusion pressure of isolated hearts from control rats (○, $n=6$) or from those treated *in vivo* with lipopolysaccharide (LPS, 10 mg kg^{-1}, i.v.) in the presence of vehicle (■, $n=6$) or TNF-α-Ab (▲, 3 mg kg^{-1}, i.v., 30 min before LPS, $n=4$). Each point with a vertical bar represents the mean±s.e.mean of n determinations. *$P<0.05$.

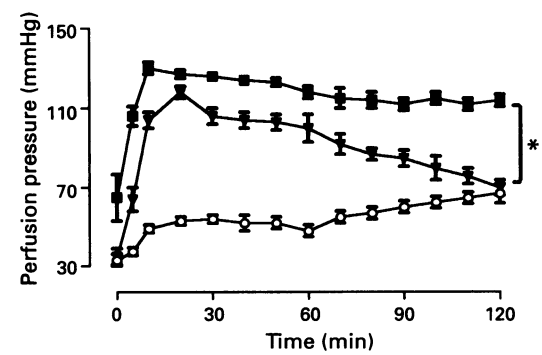

Figure 6 Changes in perfusion pressure of isolated hearts from control rats (○, $n=6$) and from those treated *in vivo* with lipopolysaccharide (LPS, 10 mg kg^{-1}, i.v.) in the presence of vehicle (■, $n=6$) or FR 139317 (▼, 0.3 mg kg^{-1} min^{-1}, i.v., $n=6$). FR 139317 (1 μM) was also present in the perfusate. Each point with a vertical bar represents the mean±s.e.mean of n determinations. *$P<0.05$.

elevation in perfusion pressure was seen in hearts removed 60 min after the injection of LPS. This suggests that the mechanism leading to coronary vasoconstriction became activated between 15 and 60 min after LPS injection and required several hours fully to develop. Such a time course would be consistent with the time required for the *de novo* synthesis and release of TNF-α (Beutler *et al.*, 1985).

Secondly, pretreatment with cycloheximide, which largely inhibits the release of TNF-α by monocytes (Hofsli *et al.*, 1988) and of ET-1 by endothelial cells (Schini *et al.*, 1989), completely abrogated the action of LPS. At the same time it must be noted, however, that we did not find dexamethasone to reduce the LPS-induced increase in coronary perfusion pressure, despite earlier reports that glucocorticoids inhibit LPS-induced cytokine release (Foster *et al.*, 1993; Ulich *et al.*, 1990) and the previous demonstration that dexamethasone treatment of rats, using the same protocol as in the present study, effectively inhibits shock-induced induction of NO synthase (Szabó *et al.*, 1993). The lack of activity in the present study agrees, however, with the limited success of glucocorticoids in the treatment of clinical septic shock (see Sheagren, 1991) and indicates that glucocorticoids may affect various consequences of septic shock by a different extent or time course.

As a third line of supportive evidence, the plasma level of TNF-α markedly increased between 15 and 60 min after the injection of LPS, a time-course which correlates very closely with the increase in coronary perfusion pressure induced by LPS. Furthermore, this increase was greatly depressed by cycloheximide, which prevented the subsequent increase in coronary perfusion pressure. Notably, the increase in circulating TNF-α was not affected by dexamethasone, which also failed to modify the coronary vasoconstriction.

The fourth, and strongest line of evidence supporting a role for TNF-α, is supplied by the marked inhibitory effect of TNF-α-Ab on the LPS-induced rise in coronary resistance. This is consistent with the beneficial activity of similar doses of anti-TNF-α antibodies in models of septic shock in mice (Silva *et al.*, 1990), pigs (Windsor *et al.*, 1994) and monkeys (Fiedler *et al.*, 1992). In human sepsis, anti TNF-α therapy did not clearly improve survival (see Natanson *et al.*, 1994), although positive clinical data have been obtained (Vincent *et al.*, 1992).

In the present study, TNF-α could not be the final mediator of the coronary vasoconstriction, for it did not affect the coronary perfusion pressure when applied directly into the perfusate *in vitro* (data not shown). As outlined above, this role was most likely filled by ET-1. Thus, ET-1 levels in the plasma were greatly elevated following injection of LPS, as has been shown before in a number of species, including the dog (Nakamura *et al.*, 1991), rat (Sugiura *et al.*, 1989; Vemulapalli *et al.*, 1991; Pollock *et al.*, 1993; Morise *et al.*, 1994), pig (Lundberg *et al.*, 1991; Myhre *et al.*, 1993) and human (Voerman *et al.*, 1992). However, our findings extend these previous observations in that they strongly suggest that TNF-α is the stimulator of this release. This conclusion would agree with findings from experiments using endothelial cells in culture, which demonstrate that exposure to TNF-α increases the transcriptional rate of the ET-1 gene (Marsden & Brenner, 1992). More recently, a study performed with pigs subjected to septic shock by infusion of live bacteria demonstrated a shock-induced increase of plasma TNF-α and endothelin (Han *et al.*, 1994). The rise in endothelin was obviously caused by TNF-α in this study, for it could nearly be prevented by pretreatment with an antibody directed against TNF-α. Our present work is in excellent agreement with the data published by Han and coworkers, and extends them by showing that the shock-induced formation (or release) of TNF-α and ET-1 is (i) associated with an increase of coronary vascular resistance, (ii) is largely (though not completely) antagonized by an ET_A receptor antagonist and (iii) can be reproduced in other species than pigs.

The suggetion that ET-1 is the mediator of the coronary vasoconstriction is also greatly supported by our finding that combined *in vivo* and *in vitro* treatment with the endothelin ET_A receptor-selective antagonist FR 139317 significantly reduced the increase in perfusion pressure in hearts removed from LPS-treated rats. This is consistent with the observation that the coronary vasculature of rats expresses the ET_A receptor subtype (Hori *et al.*, 1992). Interestingly, FR 139317 did not return the coronary perfusion pressure to control levels, particularly during the first 60 min of the *ex vivo* perfusion indicating that other vasoconstrictor mediators are also involved. However, these may well have a shorter duration of action than ET-1, for FR 139317 gave the greatest reduction in perfusion pressure (>70%) after 120 min of *ex vivo* perfusion to a level that was not different to that seen in hearts taken from control animals. It may be suggested that this vasoconstriction was in fact caused by an excessive change in myocardial wall tension or inflammatory oedema. However, this is unlikely for LPS treatment did not affect left ventricular contractility, tissue water content, or leukocyte accumulation, as assessed by light microscopy. In addition, an enhanced production of contractile eicosanoids such as thromboxane A_2 did not underly the effect, for the coronary vasoconstriction was not affected by treatment with indomethacin.

Coronary vasoconstriction induced by the release of TNF-α and ET-1 is not exclusively restricted to an inflammatory response caused by administration of LPS. We have recently shown that intravenous administration of cytokines, such as interleukin-2, also causes coronary vasoconstriction in *ex vivo* perfused rat hearts (Klemm *et al.*, 1995a). In addition, chronic inflammation, such as avidin-induced polyarthritis, may also increase coronary vascular resistance (Klemm *et al.*, 1995b). Certainly, there are differences in time course and extent of inflammatory mediator release and coronary vasoconstriction in comparison to LPS-induced shock. It is important to note, however, that in these studies endothelin receptor antagonists also reduced, or even prevented, coronary vasoconstriction.

In summary, we have shown that LPS treatment of rats induces the production of TNF-α which may increase the production and/or release of ET-1, leading to a marked and long lasting coronary vasoconstriction. As LPS may also induce the increased production of potent vasodilator mediators, including nitric oxide and prostacyclin, it is possible that the TNF-α/ET-1-mediated coronary vasoconstriction is compensated for in time. However, our data would predict that in the absence of these mediators or when the production of endogenous vasodilators is impaired, e.g. following endothelial injury or inappropriate pharmacological manipulations, there could well be a pronounced coronary spasm leading to severe myocardial ischaemia.

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