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Intrarenal haemodynamics and renal dysfunction in endotoxaemia: effects of nitric oxide synthase inhibition

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1 This study investigated the effects of low dose endotoxin (lipopolysaccharide, LPS) on (i) systemic haemodynamics, (ii) renal blood flow (RBF), (iii) renal cortical and medullary perfusion and (iv) renal function in the anaesthetized rat. We have also investigated the effects of nitric oxide (NO) synthase (NOS) inhibition with N^G-methyl-L-arginine (L-NMMA) on the alterations in systemic and renal haemodynamics and renal function caused by endotoxin.

2 Infusion of low dose LPS (1 mg kg^{-1} over 30 min, $n=6$) caused a late fall in mean arterial blood pressure (MAP, at 5 and 6 h after LPS), but did not cause an early (at 1–4 h after LPS) hypotension. The pressor effect of noradrenaline (NA, 1 μg kg^{-1}, i.v.) was significantly reduced at 1 to 6 h after LPS (vascular hyporeactivity). Infusion of L-NMMA (50 μg kg^{-1} min^{-1} commencing 60 min before LPS and continued throughout the experiment, $n=7$) abolished the delayed hypotension and significantly attenuated the vascular hyporeactivity to NA (at 2–6 h).

3 Infusion of LPS (1 mg kg^{-1} over 30 min, $n=6$) caused a rapid (within 2 h) decline in renal function (measured by inulin clearance) in the absence of a significant fall in MAP or renal blood flow (RBF). L-NMMA ($n=7$) attenuated the impairment in renal function caused by LPS so that the inulin clearance in LPS-rats treated with L-NMMA was significantly greater than in LPS-rats treated with vehicle (control) at 3–6 h after infusion of LPS.

4 Endotoxaemia also caused a significant reduction in renal cortical, but not medullary perfusion (measured as Laser Doppler flux). Infusion of L-NMMA caused a significant further fall in cortical perfusion and a significant fall in medullary perfusion in the absence of changes in RBF.

5 Infusion of LPS resulted in a progressive increase in the plasma levels of nitrite/nitrate (an indicator of the formation of NO), so that the plasma concentration of nitrite/nitrate was significantly higher than baseline at 150 to 330 min after LPS. Infusion of L-NMMA attenuated the rise in the plasma concentration of nitrite/nitrate (at 270 and 330 min, $P<0.05$) caused by LPS.

6 Thus, the renal dysfunction caused by injection of low dose of endotoxin in the rat occurs in the absence of significant falls in blood pressure or total renal blood flow. Inhibition of NOS activity with L-NMMA attenuates the renal dysfunction caused by endotoxin (without improving intrarenal haemodynamics), suggesting that an overproduction of NO may contribute to the development of renal injury and dysfunction by causing direct cytotoxic effects.

Keywords: Nitric oxide; endotoxaemia; acute renal failure; renal blood flow

Introduction

Septic shock is the leading cause of death in non-coronary intensive care units today. The development of acute renal failure (ARF) is an ominous sign associated with an increased morbidity and mortality (Cameron, 1990; Rayner *et al*., 1990). Despite advances in the supportive treatment of ARF in sepsis, the mortality from septic shock has not changed appreciably over the past 20 years. The pathophysiology of the renal dysfunction in sepsis remains incompletely understood, but factors include ischaemic renal injury from hypotension, injury mediated by activated leukocytes and humoral factors, as well as direct injury to the tubule epithelial cells from endotoxin (see Millar & Thiemermann, 1997 for review). In addition, an imbalance between the heterogeneous intrarenal distribution of perfusion and metabolic demand of different tubule segments can lead to regional ischaemia in focal areas of the kidney (Brezis *et al*., 1984; Brezis & Rosen, 1995). Although current conservative management of patients with ARF aims to optimize overall renal perfusion and to limit its energy expenditure, there is a little consensus as to its efficacy. Thus, a greater understanding of the role of alterations in intrarenal blood flow distribution, as well as the mechanism of the direct cytotoxic effects of endotoxin, in the pathophysiology of the renal dysfunction associated with sepsis may help to design more rational therapies to improve the outcome of this syndrome.

An overproduction of the free radical nitric oxide (NO) has been implicated in the circulatory dysfunction and organ failure that occurs in endotoxic shock (Thiemermann, 1995). However, the contribution of endogenous NO to the renal dysfunction caused by endotoxin is largely unclear. All three isoforms of NO synthase (NOS), namely neuronal (nNOS or NOS I), inducible (iNOS or NOS II) and endothelial (eNOS or NOS III) isoforms are expressed in the normal kidney (Mundel *et al*., 1992; Wilcox *et al*., 1992; Morrisey *et al*., 1994; Tojo *et al*., 1994; Bachmann *et al*., 1995). Endotoxaemia causes the expression within the kidney of iNOS mRNA and protein (Markewitz *et al*., 1993; Morrisey *et al*., 1994), increases the activity of eNOS (Mayeux *et al*., 1995), but does not affect the expression of nNOS (Tojo *et al*., 1994). Although endogenous NO plays an important role in the control of regional blood flow and intrarenal haemodynamics, (e.g. in supporting medullary perfusion) (Brezis *et al*., 1991; Walder *et al*., 1991; Mattson *et al*., 1992), the contribution of NO to changes in renal function following injury is still relatively poorly understood. There is evidence that inhibition of NOS activity with (a high dose of) N^G-nitro-L-arginine methyl ester (L-NAME), a relatively selective inhibitor of eNOS activity in cultured cells (Southan *et al*., 1995) as well as in the anaesthetized rat (Wu *et al*., 1996), reduces renal function in rats with or without endotoxaemia (Schultz & Raij, 1992). In addition, L-NAME augments the degree of glomerular thrombosis caused by lipopolysaccharide (LPS, endotoxin) in this species (Shultz &

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Raij, 1992). The hypothesis that inhibition of NOS activity may be deleterious is further supported by studies documenting that inhibition of NOS activity in rodents with endotoxaemia reduces renal perfusion (Wright *et al*., 1992; Henderson *et al*., 1994; Robertson *et al*., 1994; Spain *et al*., 1994). In contrast, inhibition of NOS activity (L-NMMA) in sheep with endotoxic shock which had been sufficiently resuscitated with fluids, (i) did not lead to a reduction in renal blood flow below baseline and (ii) increased urine output (Booke *et al*., 1996).

This study was designed to evaluate the role of NO in the pathophysiology of the renal dysfunction caused by endotoxin in the rat. Specifically, in this study (i) the alterations in systemic and intrarenal haemodynamics were compared with those in renal function caused by endotoxaemia and (ii) the effects of inhibition of NO synthesis on haemodynamic and functional parameters were investigated.

Methods

Surgical preparation

Male Wistar rats weighing 248–364g (Tuck, Rayleigh, Essex, U.K.) were anaesthetized with thiopentone sodium (100 mg kg^{-1}, i.p.) and placed on a thermostatically controlled heating mat (Harvard Apparatus Ltd., Edenbridge, Kent, U.K.). All animals were cared for in accordance with the Home Office 'Guidance in the operation of the Animals (Scientific Procedures) Act 1986'. Polyethylene cannulae (PP50, I.D. 0.58 mm, Portex, Hythe, Kent, U.K.) were placed in the carotid artery and both jugular veins, the bladder was cannulated (PP90, I.D. 0.76 mm), and a tracheostomy was fashioned to maintain airway patency and facilitate spontaneous respiration. Through a flank incision, the left kidney was mobilized and supported in a perspex cup to eliminate respiratory movement. The left ureter was cannulated (PP25, I.D. 0.40 mm), tied and divided distally. An ultrasonic flow probe was placed around the left renal artery and connected to a Doppler ultrasound flowmeter (T206, Transonics, Ithaca, NY., U.S.A.). A surface Laser Doppler probe (PF318, Perimed UK Ltd., Bury St Edmunds, U.K.) was placed directly over the renal cortex, and a needle Laser Doppler probe with an outside diameter of 0.45 mm (PF 302) was inserted through the cortex into the renal medulla to a depth of 3.0–4.0 mm from the renal surface. Both probes were connected to Laser Doppler flowmeters (PF3, Perimed, UK Ltd, Bury St Edmunds, U.K.). The carotid arterial cannula was connected via a pressure transducer (Senso-Nor 840, Senso-Nor, Horten, Norway) to a data acquistion system (MacLab 8e, ADInstruments, Hastings, U.K.) installed on an Apple Macintosh computer, thus permitting measurement of arterial pressure and derivation of heart rate from the pulse waveform, and line outputs from the ultrasound and Laser Doppler flowmeters were also connected to the MacLab.

At the end of all experiments, animals were killed by an overdose of thiopentone sodium, whereupon background flux values for cortex and medulla were measured and then subtracted from previous recordings, to eliminate non-specific effects of laser reflection. The left kidney was then dissected and the medullary position of the needle probe was verified by inspection.

Clearance of ^{3}H-labelled inulin

At the end of the surgical preparation, a bolus of [^{3}H]-inulin (5μCi in NaCl of 0.9% 0.5 ml kg^{-1}, i.v.) was administered, followed by a constant infusion at a rate of 1.5 μCi h^{-1} in NaCl 0.9% at 1.5 ml h^{-1}. Rats also received a co-infusion of vehicle (NaCl 0.9% at 1.5 ml kg^{-1}). Following equilibration for 1 h, urine was collected continuously at hourly intervals from the bladder cannula and ureteric cannula, and plasma samples were obtained at the mid-point of each clearance period, thus permitting measurement of renal function from the right and left kidney independently. This was used to ensure that the left kidney was not functionally compromised by the surgical preparation. In cases ($n=2$) where inulin clearance from the left kidney was significantly different (e.g. reduced by more than 40%) from the right (non-instrumented) kidney, animals were excluded from the experiment. In all other animals, the activity of [^{3}H]-inulin in plasma and urine (from both kidneys) was measured in a scintillation counter (Beckman Instrumentation, Fullerton, CA, U.S.A.) and inulin clearance was calculated by use of standard formulae.

Vasopressor response to noradrenaline (NA response)

At the end of each hourly clearance period, noradrenaline (1 μg kg^{-1}, i.v.) was administered as a bolus injection and the resulting increase in mean arterial pressure (MAP) was recorded (NA response).

Effects of LPS on renal function and haemodynamics

Following equilibration, baseline haemodynamics, including MAP, heart rate, total renal blood flow to the left kidney (RBF), cortical and medullary Laser Doppler flux, as well as NA response were recorded. Urine was collected over two sequential hours with midpoint plasma samples to calculate inulin clearance. Thereafter, a small dose of LPS (1 mg kg^{-1}) was infused in 0.45 ml NaCl 0.9% over 30 min ($n=6$). Haemodynamic parameters and inulin clearance were recorded for a further 6 h following the induction of endotoxaemia.

Effect of NOS inhibition on renal dysfunction and haemodynamics in endotoxaemia

This study group followed the same protocol as described above for the LPS group, except that animals ($n=7$) received an infusion of the NOS inhibitor, N^G-methyl-L-arginine (L-NMMA, 50 μg kg^{-1} min^{-1} in NaCl 0.9% at a rate of 1.5 ml h^{-1}). This replaced the NaCl co-infusion in control animals, and was commenced 60 min before the induction of endotoxaemia, thus permitting assessment of the independent effects of NOS inhibition (in the absence of endotoxaemia) on renal haemodynamics and function in this model.

Effect of NOS inhibition on renal function and haemodynamics in rats without endotoxaemia (control)

In order to assess the time-dependent changes in any of the parameters measured, one group of rats ($n=4$) received an infusion of vehicle (saline 1.5 ml h^{-1}) and was monitored for 8 h. In order to assess the effects of NOS inhibition (in the absence of endotoxaemia) on any of the parameters measured, one further group of animals ($n=5$) received an infusion of L-NMMA, (50 μg kg^{-1} min^{-1} in NaCl 0.9% at a rate of 1.5 ml h^{-1}), which started 60 min before the administration of saline (0.45 ml; rather than LPS) and was continued for a further 7 h. As all of these animals also received an infusion of inulin (see above), the total volume of saline infused was 3 ml h^{-1}.

Measurement of plasma nitrite and nitrate

Nitrite and nitrate are the primary oxidation products of NO reacting with oxygen and, therefore, the nitrite/nitrate concentration in plasma was used as an indicator of NO synthesis. Blood was collected into heparine-treated capillary tubes and centrifuged (6,000 r.p.m. for 5 min) to separate cells and plasma. Then, the nitrate in the plasma sample was enzymatically converted to nitrite according to the method of Schmidt *et al*. (1992). Briefly nitrate was stoichiometrically reduced to nitrite by incubation of sample aliquote (25 μl) for 15 min at 37°C, in the presence of nitrate reductase (1 iu ml^{-1}, E.C. 1.6.6.2), NADPH (500 μM) and flavine adenine dinucleotide (FAD, 50 μM) in a final volume of 40µl. When nitrate reduction was complete, the unused NADPH, which interferes

with the subsequent nitrite determination, was oxidized by lactate dehydrogenase (100 iu ml^{-1}) and sodium pyruvate (100 mM) in a final reaction volume of 50 μl and incubated for 5 min at 37°C. Subsequently, the total nitrite in the plasma was assayed by adding 50 μl of Griess reagent (4% sulphanilamide and 0.2% naphtylenediamide in 10% phosphoric acid) to each sample (Green *et al*., 1981). The optical density at 550 nm (OD_{550}) was measured by a Molecular Devices microplate reader (Richmond, CA, U.S.A.). Total nitrite/nitrate concentrations were calculated by comparison with OD_{550} of a standard solution of sodium nitrate (also stoichiometrically converted to nitrite) prepared in saline.

Materials

[^{3}H]inulin was obtained from Amersham Life Sciences (Little Chalfont, Bucks, U.K.), and thiopentone sodium was obtained from Rhône Mérieux (Harlow, Essex, U.K.). Nitrate reductase (from *Aspergillus species*) and lactate dehydrogenase (from rabbit muscle) were obtained from Boehringer-Mannheim (Nottingham, U.K.). Lipopolysaccharide (*Escherichia Coli* serotype 0127:B8) and all other chemicals/materials were from Sigma Chemical Company (Poole, Dorset, U.K.). All drugs were dissolved in NaCl 0.9%.

Statistical analysis

All values in the figures and text are expressed as mean ± s.e.mean of n observations, where n is the number of rats in each experimental group. The data were analysed by use of a factorial or repeated measures ANOVA followed by Scheffe's test or unpaired Student's t test where appropriate. A P value of less than 0.05 was considered to be statistically significant.

Results

Alterations in systemic haemodynamics following LPS: effects of nitric oxide synthase inhibition

Baseline values for MAP or heart rate did not differ between the four groups studied (Table 1). Infusion of saline in rats which had not received LPS (time control) did not cause a significant change in any of these parameters (Table 2). In rats without endotoxaemia, infusion of L-NMMA also had no significant effect on any of these parameters (Table 2). Thus, there was no difference in MAP or heart rate between those two groups of animals ($P>0.05$, Table 2).

Infusion of LPS (1 mg kg^{-1}, $n=6$) did not cause an acute fall in MAP. Between 4 and 6 h after injection of LPS, there was a significant fall in MAP (when compared with baseline data for MAP) (Figure 1a). Infusion of LPS resulted within 2 h in a significant increase in heart rate, and heart rate remained significantly elevated until the end of the experiment (Figure 1b). Infusion of LPS also attenuated the pressor responses elicited by NA (at 1 to 6 h after LPS, $P<0.05$, $n=6$; Figure 1c).

Infusion of L-NMMA (50 μg kg^{-1} min^{-1}, $n=7$) starting 1 h before the infusion of LPS did not cause a significant alteration in MAP (Figure 1a), heart rate (Figure 1b) or the pressor response elicited by NA (Figure 1c). However, the infusion of L-NMMA prevented the delayed fall in MAP, but did not affect the tachycardia, caused by endotoxaemia in the rat (Figure 1a and b). Infusion of L-NMMA did not attenuate the vascular hyporeactivity to NA which developed within 1 h, but reduced the degree of vascular hyporeactivity to NA which occurred between 2 and 6 h after infusion of LPS. Thus, the pressor responses elicited by NA were significantly greater in LPS-rats treated with L-NMMA (at 2,3,4 and 6 h after infusion of LPS, $P<0.05$, $n=7$) than the ones elicited by NA in LPS-rats treated with vehicle ($n=6$; Figure 1c).

Alterations in renal haemodynamics following LPS: effects of nitric oxide synthase inhibition

Baseline values for RBF did not differ between the four experimental groups (Table 1). Infusion of saline in rats which had not received LPS (time control) did not cause a significant change in RBF, cortical flux or medullary flux (Table 2). In rats without endotoxaemia, infusion of L-NMMA for 7 h also had no significant effect on any of these parameters (when compared to baseline; see Table 2). Thus, there was no difference in cortical or medullary flux between those two groups of animals ($P>0.05$, Table 2). Surprisingly, at one time point (e.g. 360 min) the RBF in rats treated with L-NMMA was slightly, but significantly lower than in rats which had received saline rather than L-NMMA (Table 2).

Infusion of LPS did not cause a significant change in RBF ($P>0.05$, $n=6$, Figure 2a). Although infusion of LPS also did not cause an immediate fall in cortical flux (within the first 1 h of endotoxaemia), cortical flux had fallen by 20 ± 3% from the baseline by 2 h after the start of the infusion of LPS. In these animals, cortical flux remained significantly reduced from 2 to 6 h after LPS ($P<0.05$, when compared to baseline, $n=6$;

Table 1 Baseline values for mean arterial pressure (MAP), heart rate (HR), renal blood flow (RBF) and inulin clearance (C_{in})

	MAP (mm Hg)	*HR* (beats min^{-1})	*RBF* (ml min^{-1})	C_{in} (ml min^{-1} $100\,g^{-1}$)
Sham	117 ± 7	350 ± 12	10.8 ± 0.7	1.02 ± 0.2
L-NMMA	117 ± 5	351 ± 14	9.9 ± 0.5	1.18 ± 0.09
LPS	112 ± 3	333 ± 11	9.3 ± 0.4	1.17 ± 0.05
L-NMMA + LPS	110 ± 2	338 ± 7	9.5 ± 0.8	1.05 ± 0.08

Data shown are means ± s.e.mean

Table 2 Haemodynamic and functional parameters over time in sham-operated and L-NMMA control rats

		Timepoint −60 min	0 min	360 min
MAP (mmHg)	Vehicle	117 ± 7	113 ± 5	111 ± 7
	L-NMMA	117 ± 5	117 ± 4	107 ± 5
HR (beats min^{-1})	Vehicle	350 ± 12	343 ± 8	361 ± 17
	L-NMMA	352 ± 14	349 ± 15	348 ± 5
RBF (ml min^{-1})	Vehicle	10.8 ± 0.7	10.7 ± 0.8	12.5 ± 0.7
	L-NMMA	9.9 ± 0.5	10 ± 0.6	9.1 ± 0.5*
Cortical flux (% change)	Vehicle	0	0.8 ± 1	−6 ± 9
	L-NMMA	0	0.4 ± 1.6	−6 ± 5.4
Medullary flux (% change)	Vehicle	0	−1.5 ± 0.6	10.7 ± 5.5
	L-NMMA	0	−1.4 ± 1.8	9.4 ± 6.2
C_{in} (% baseline)	Vehicle	100	111 ± 8	108 ± 10
	L-NMMA	100	91 ± 7	85 ± 7

Data shown are means ± s.e.mean. *$P<0.05$ vehicle vs L-NMMA. For abbreviations used, see Table 1.

Figure 2b). In rats which had received an injection of LPS, medullary flux tended to increase (maximum at 60 min). This was followed by a fall in medullary flux of approximately 20% (Figure 2c, $P>0.05$ when compared to baseline). Infusion of L-NMMA exacerbated the fall in cortical flux caused by endotoxin ($P<0.05$ when compared to rats treated with LPS plus vehicle, $n=7$; Figure 2b). The fall in medullary flux caused by infusion of L-NMMA before the infusion of LPS was followed by a significant increase in medullary flux, which occurred in the first hour after the start of the infusion of LPS. Thereafter, medullary flux fell progressively and was significantly lower than baseline at 3 to 6 h ($P<0.05$ when compared to baseline, $n=7$, Figure 2c).

Alterations in renal function following LPS: effects of nitric oxide synthase inhibition

Baseline values for inulin clearance did not differ between the four experimental groups studied (Table 1). Infusion of saline in rats which had not received LPS (time control) caused an increase of $\sim 10\%$ in inulin clearance, which was not significant when compared to baseline (Table 2). In rats without endotoxaemia, infusion of L-NMMA for 7 h resulted in a 15%

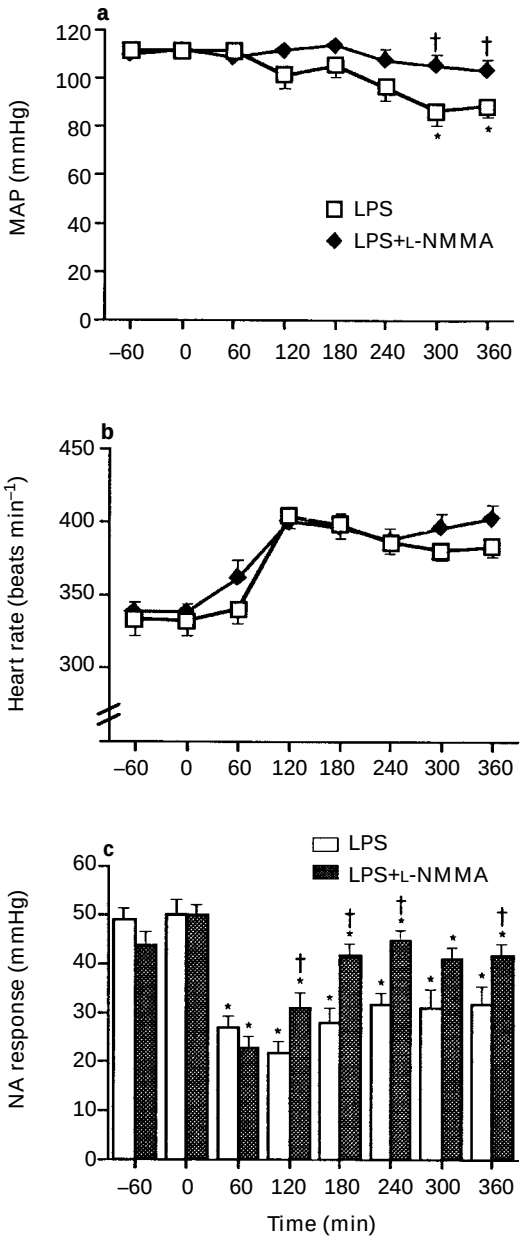

Figure 1 Changes in mean arterial blood pressure (MAP, a), heart rate (b) and pressor response elicited by noradrenaline (NA, 1 μg kg^{-1}, i.v.; c) in rats subjected to infusion of endotoxin (lipopolysaccharide, LPS, 1 mg kg^{-1} over 30 min) and treated with an infusion of either vehicle (saline, open symbols and columns, $n=6$) or the nitric oxide synthase inhibitor N^{G}-methyl-L-arginine (L-NMMA, 50 μg kg^{-1} min^{-1} commencing 60 min before LPS and continued throughout the experiment; solid symbols and columns; $n=7$). *$P<0.05$ when compared to baseline measured 1 h before infusion of LPS (−1 h); †$P<0.05$ when compared to LPS-treated rats at the same time point.

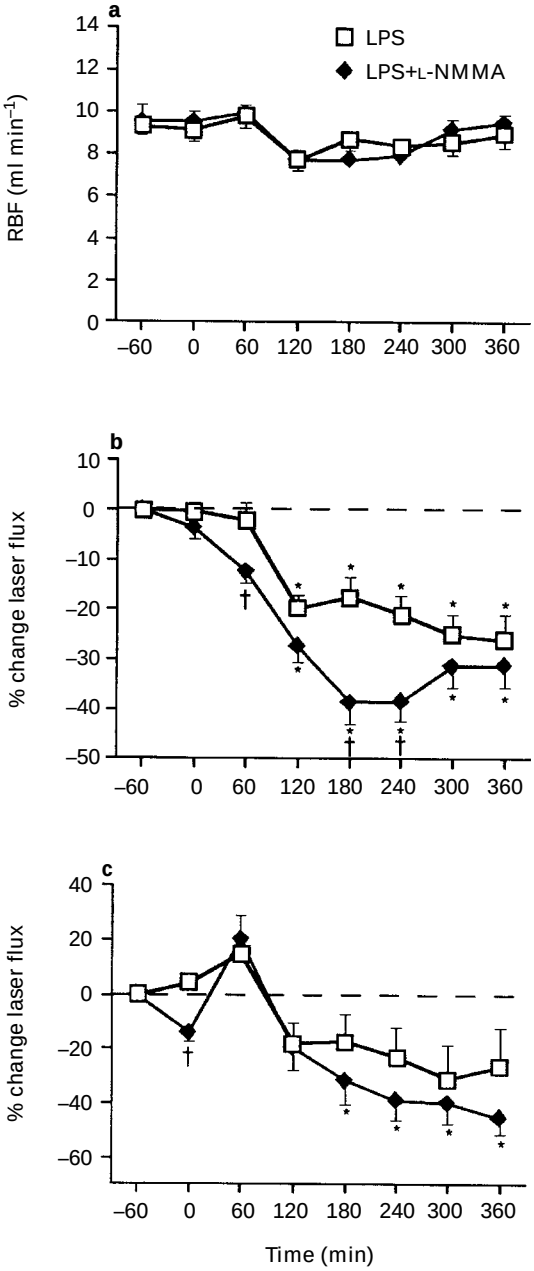

Figure 2 Changes in renal blood flow (RBF, a), cortical Laser Doppler flux (b) and medullary Laser Doppler flux (c) in rats subjected to infusion of endotoxin (lipopolysaccharide, LPS, 1 mg kg^{-1} over 30 min) and treated with an infusion of either vehicle (saline, open symbols, $n=6$) or the nitric oxide synthase inhibitor N^{G}-methyl-L-arginine (L-NMMA, 50 μg kg^{-1} min^{-1} commencing 60 min before LPS and continued throughout the experiment; solid symbols; $n=7$). *$P<0.05$ when compared to baseline measured 1 h prior to infusion of LPS (−1 h); † when compared to LPS-treated rats at the same time point.

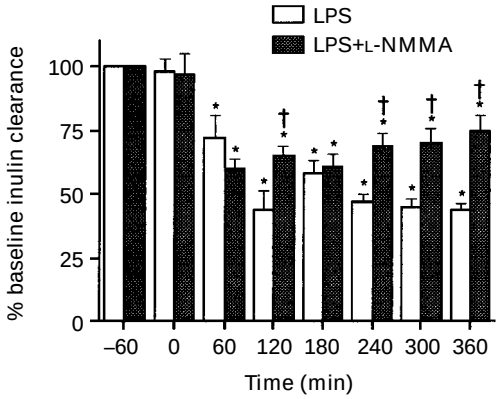

Figure 3 Changes in inulin clearance in rats subjected to infusion of endotoxin (lipopolysaccharide, LPS, 1 mg kg^{-1} over 30 min) and treated with an infusion of either vehicle (saline, $n=6$) or the nitric oxide synthase inhibitor N^G-methyl-L-arginine (L-NMMA, 50 $\mu g\ kg^{-1}\ min^{-1}$ commencing 60 min before LPS and continued throughout the experiment; $n=7$). $^*P<0.05$ when compared to baseline measured 1 h before infusion of LPS (−1 h); †$P<0.05$ when compared to LPS-treated rats at the same time point.

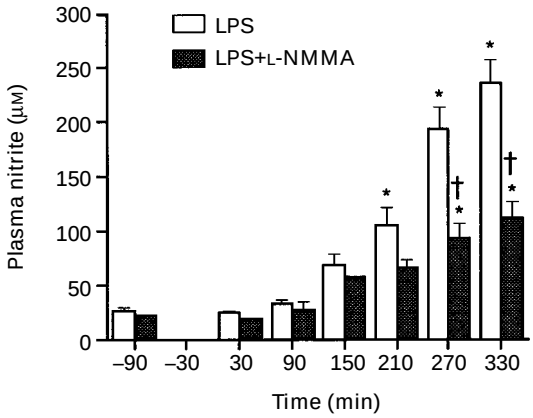

Figure 4 Increase in the plasma levels of nitrite/nitrate in rats subjected to infusion of endotoxin (lipopolysaccharide, LPS, 1 mg kg^{-1} over 30 min) and treated with an infusion of either vehicle (saline, $n=6$) or the nitric oxide synthase inhibitor N^G-methyl-L-arginine (L-NMMA, 50 $\mu g\ kg^{-1}\ min^{-1}$ commencing 60 min before LPS and continued throughout the experiment; $n=7$). $^*P<0.05$ when compared to baseline measured 1 h before infusion of LPS (−1 h); †$P<0.05$ when compared to LPS-treated rats at the same time point.

fall in inulin clearance, which was also not significant when compared to baseline (Table 2). However, the clearance of inulin was (at 360 min) significantly lower in rats which had received an infusion of L-NMMA rather than saline ($P<0.05$, Table 2).

Within 2 h after the commencement of the infusion of LPS, inulin clearance fell to 44±7% of baseline clearance ($P<0.05$ compared to baseline), and remained significantly reduced until the end of the experiment at 6 h (Figure 3). In LPS-rats which received L-NMMA, the fall in inulin clearance which occurred within the first h after the start of the infusion of LPS was similar. However, L-NMMA prevented the further decline in inulin clearance (observed in rats treated with LPS plus vehicle), so that the mean values for inulin clearance were significantly greater in LPS-rats treated with L-NMMA than in rats treated with LPS and vehicle ($P<0.05$ at 1–2, 3–4, 4–5 and 5–6 h after LPS when compared to LPS-control, $n=7$; Figure 3).

Increase in plasma nitrite/nitrate caused by LPS: effects of nitric oxide synthase inhibition

Infusion of LPS resulted in a progressive increase in the plasma levels of nitrite/nitrate (an indicator of the formation of NO), so that the plasma concentration of nitrite/nitrate was significantly higher than baseline at 150 to 330 min after LPS ($P<0.05$, $n=6$). Infusion of L-NMMA attenuated the rise in the plasma concentration of nitrite/nitrate ($P<0.05$ at 270 and 330 min, $n=7$) caused by LPS (Figure 4).

Discussion

This study demonstrated that a small dose of endotoxin, which does not cause a significant (early) fall in blood pressure or RBF, causes a substantial impairment of renal function as measured by inulin clearance in the anaesthetized rat. These effects were specifically due to endotoxaemia, as (i) systemic haemodynamics, (ii) intrarenal haemodynamics and (iii) renal function were stable throughout the 7 h experimental period in rats which had received saline rather than LPS. In LPS-rats, inhibition of NOS activity with L-NMMA reduced the degree of renal dysfunction and prevented the delayed hypotension (occurring at 5 to 6 h after injection of endotoxin) caused by low dose endotoxin. In rats without endotoxaemia, an identical infusion of L-NMMA (for 7 h) did not cause any significant alterations in haemodynamics (systemic or renal) or renal function (when compared to baseline). These findings suggest that the dose regimen of L-NMMA chosen did not result in a substantial inhibition of eNOS activity (in the kidney).

Our finding that a greater than 50% fall in renal function occurred in the absence of significant falls in either blood pressure or renal blood flow (and hence renal perfusion pressure) demonstrates that there is no correlation between total renal blood flow and function in the absence of changes in blood pressure. This finding also suggests that the impairment in renal function observed in this study is secondary to either alterations in intrarenal perfusion or factors which are independent of any haemodynamic changes. Indeed, infusion of endotoxin was associated with a rapid (within 2 h) decline in perfusion of the renal cortex (measured as cortical Laser Doppler flux) as well as a minor fall in the perfusion of the renal medulla, without a fall in total RBF. This finding suggests that endotoxaemia causes an increase in arterio-venous shunting resulting in a fall in tissue perfusion. There is evidence that arterio-venous shunting occurs even in normal kidneys probably at the level of the arcuate and/or interlobular vessels (Schurek *et al*., 1990). Our hypothesis, that an increase in the degree of pre-capillary shunting within the kidney can lead to a reduction in tissue perfusion, is supported by the finding that hyperdynamic sepsis in the sheep results in an increase in total renal arterial blood flow without a significant change of renal perfusion (measured by radioactive or coloured microspheres) (Booke *et al*., 1995). In addition to the development of pre-capillary shunts, renal (tissue) perfusion may be further compromised by endotoxin-induced alterations in blood rheology, including a decrease in red cell deformability (Hurd *et al*., 1987; Puranapanda *et al*., 1987), or increases in blood viscosity (Chien, 1982) or vascular permeability (Groenveld & Thijs, 1987).

We demonstrated here that infusion of the NOS inhibitor L-NMMA worsened the fall in cortical and medullary perfusion caused by low dose endotoxin in the anaesthetized rat, without causing a significant fall in RBF (when compared to LPS-rats which had received vehicle). Surprisingly, this further reduction in renal perfusion caused by L-NMMA was associated with an improvement in renal function. What, then, is the mechanism by which L-NMMA attenuates the renal dysfunction caused by endotoxin in the rat? Although L-NMMA attenuated the delayed fall in blood pressure caused by endotoxin, an improvement in renal perfusion does not account for the beneficial effect of this inhibitor of NOS activity, as L-NMMA significantly reduced, rather than improved, re-

nal perfusion. Thus, we propose that enhanced formation of NO in endotoxaemia results in a direct injury to the nephron. There is good evidence that enhanced formation of NO (particularly following the induction of iNOS) can cause cytotoxic effects. Indeed, large amounts of NO cause autoinhibition of mitochondrial respiration by inhibiting several key enzymes in the mitochondrial respiratory chain (NADH-ubiquinone reductase, succinate-ubiquinone oxidoreductase) or in the Krebs' cycle (e.g. cis-acconitase) resulting in a shift in glucose metabolism from aerobic to anaerobic pathways (Morris & Billiar, 1994; Thiemermann, 1995). NO also causes DNA strand breakage which triggers a futile, energy-consuming repair cycle by activating the nuclear enzyme poly(ADP)ribosyltransferase (PARS), which results in the depletion of the intracellular concentration of NAD^+ (its substrate) and ATP and ultimately cell death (Schraufstatter *et al*., 1986; Zingarelli *et al*., 1996). Furthermore, NO mediates the injury caused by hypoxia in isolated proximal tubule cells. The release of NO caused by hypoxia in these cells precedes the release of lactate dehydrogenase (LDH) and, hence, cell membrane damage and inhibition of NOS activity attenuates LDH release (Yu *et al*., 1994; Yaqoob *et al*., 1996). Taken together, these studies support the view that an overproduction of NO in the kidney can, in principle, cause toxic effects that lead to cell injury and, hence, organ dysfunction.

The question as to whether the observed overproduction of NO caused by endotoxin in this study is due to activation of eNOS or induction of iNOS is difficult to address. The enhanced formation of NO caused by a large dose of endotoxin (10 mg kg^{-1}) in the rat is due to an early (5 min to 120 min after LPS) activation of eNOS and a subsequent induction of iNOS (Szabo *et al*., 1993). Endotoxin enhances the formation of NO by activating eNOS in cultured endothelial cells (Salvemini *et al*., 1990) as well as in isolated proximal tubule cells (Mayeux *et al*., 1995). The vascular hyporeactivity (to noradrenaline) caused within 1 h by the small dose of LPS used in this study was not attenuated by L-NMMA and, hence, not due to an enhanced formation of NO by eNOS. Nevertheless, we cannot exclude the possibility that an enhanced formation of NO by eNOS (within the kidney) contributes to the rapid (within 2 h) alterations in intrarenal blood flow distribution or function caused by endotoxin. Pro-inflammatory cytokines cause the expression of iNOS protein in renal tubule cells of the rat (Markewitz *et al*., 1993) and prolonged periods of endotoxaemia in the rat lead to an increased expression of iNOS mRNA in the kidney (Morrisey *et al*., 1994). In our study, low dose endotoxaemia did not cause a significant increase in the serum levels of nitrite/nitrate within 90 min. However, from 150 min onwards there was a progressive increase in the plasma levels of nitrite/nitrate. This increase in nitrite/nitrate is due to an enhanced formation of NO by iNOS, as (i) there is a close correlation between the rise in the plasma levels of nitrite/nitrate and the expression of iNOS protein and activity (Kengatharan *et al*., 1996), (ii) inhibition of eNOS activity with L-NAME does not reduce the increase in nitrite caused by LPS in the anaesthetized rat (Wu *et al*., 1996), and the dose of L-NMMA used in this study is sufficient to inhibit iNOS activity in the lung and liver of rats with endotoxaemia (Thiemermann *et al*., 1995). Our hypothesis that an enhanced formation of NO by iNOS contributes to the (delayed) impairment in renal function is supported by our findings that (i) L-NMMA reduced the delayed (3–6 h), but not the acute (2 h) impairment in renal function, (ii) the delayed, but not the early, renal dysfunction was associated with a significant rise in the plasma levels of nitrite/nitrate, and (iii) the attenuation by L-NMMA of the renal dysfunction caused by LPS was associated with a reduction in the plasma levels of nitrite/nitrate. Furthermore, the continuous infusion of L-NMMA abolished the delayed hypotension (5–6 h) and reduced the vascular hyporeactivity to noradrenaline occurring at 2 to 6 h after infusion of LPS. Both of these features of the circulatory failure caused by LPS are due to induction of iNOS (Thiemermann *et al*., 1995).

In conclusion, this study demonstrates that the renal dysfunction caused by injection of a low dose of endotoxin in the rat occurs in the absence of significant falls in blood pressure or total renal blood flow. Inhibition of NOS activity with L-NMMA attenuated the renal dysfunction caused by endotoxin (without improving intrarenal haemodynamics), suggesting that an overproduction of NO may contribute to the development of renal injury and dysfunction by causing direct cytotoxic effects. The cellular and enzymatic source of NO (e.g. eNOS or iNOS) is unclear and warrants further investigation.

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