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Effect of selective blockade of endothelin ET_B receptors on the liver dysfunction and injury caused by endotoxaemia in the rat

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1 We investigated the effects of the selective endothelin $(ET)_A$ receptor antagonist BQ-485 and the selective ET_B receptor antagonist BQ-788 on circulatory failure, multiple organ dysfunction syndrome (MODS) and the alterations in acid base balance caused by endotoxaemia in the anaesthetized rat.

2 Male Wistar rats were anaesthetized (thiopentone sodium; 120 mg kg^{-1}, i.p.) and received a continuous infusion of vehicle (saline, 0.6 ml $kg^{-1}h^{-1}$, i.v.), BQ-485 (10 nmol kg^{-1} min^{-1}, i.v.) or BQ-788 (10 nmol kg^{-1} min^{-1}, i.v.). Fifteen min later, animals received a bolus injection of either saline (0.9% NaCl, 1 ml kg^{-1}, i.v.) or *E. coli* lipopolysaccharide (LPS, 10 mg kg^{-1}, i.v.).

3 Injection of LPS resulted in a fall in blood pressure from 115 ± 4 mmHg (time 0) to 82 ± 4 mmHg at 360 min ($n = 15$) as well as a hyporeactivity to the pressor responses to noradrenaline (NA, 1 μg kg^{-1}, i.v.). Infusion of BQ-788 attenuated the delayed hypotension (at 360 min: 100 ± 4 mmHg, $n = 7$; $P < 0.05$) and significantly enhanced the pressor responses elicited by NA (at 60 to 240 min). In contrast, treatment of LPS-rats with BQ-485 augmented the hypotension (at 360 min), but did not affect the vascular hyporeactivity elicited by endotoxaemia.

4 Endotoxaemia for 360 min resulted in rises in the serum levels of urea and creatinine (indicators of renal failure), glutamate-oxalate-transferase (GOT) and glutamate-pyruvate-transferase (GPT) (indicators of hepatocellular injury), and bilirubin and γ-glutamyl transferase (γGT) (indicators of liver failure) as well as nitrite (indicator of the induction of nitric oxide synthase; iNOS). Treatment of LPS-rats with BQ-788, but not with BQ-485, attenuated the degree of liver injury and failure, while neither BQ-788 nor BQ-485 affected the acute renal failure or the induction of iNOS caused by endotoxin.

5 Endotoxaemia also caused (within 15 min) an acute metabolic acidosis (falls in pH, HCO_3^- and base excess) which was compensated by hyperventilation (fall in $PaCO_2$). Treatment of LPS-rats with BQ-788 or BQ-485 did not affect the metabolic acidosis caused by LPS.

6 Thus, the selective ET_B receptor antagonist BQ-788 attenuated (i) the delayed hypotension, (ii) the vascular hyporeactivity to NA as well as (iii) the degree of hepatocellular injury and dysfunction caused by endotoxin in the anaesthetized rat. In contrast, the selective ET_A receptor antagonist did neither attenuate the circulatory failure nor the liver or renal dysfunction associated with endotoxaemia. We propose that the prevention of the hepatocellular dysfunction and injury caused BQ-788 in endotoxaemia is due to an improvement in oxygen delivery to the liver secondary to (i) inhibition of pre-sinusoidal constriction, (ii) inhibition of sinusoidal constriction, and (iii) improvement in perfusion pressure.

Keywords: Endotoxic shock; vascular hyporeactivity; multiple organ failure; endothelin receptors ET_A and ET_B; BQ-485; BQ-788

Introduction

Endothelin-1 (ET-1), a member of the 21-amino acid endothelin family of peptides (ET-1, ET-2, ET-3 and sarafotoxins), is a potent vasoconstrictor produced by the endothelium from its precursor big-endothelin-1 by endothelin-converting enzyme-1 (see Yanagisawa & Masaki, 1989; Warner, 1993). Two distinct endothelin receptors have been cloned and expressed, namely ET_A (Arai *et al.*, 1990) and ET_B (Sakurai *et al.*, 1990). The vasoconstrictor effects of ET-1 are primarily mediated by activation of the ET_A receptor, which is present on vascular smooth muscle cells. Activation by ET-1 of the ET_B receptor located on endothelial cells results in a release of nitric oxide (NO) and prostacyclin to cause vasodilatation (De Nucci *et al.*, 1988; Thiemermann *et al.*, 1989). ET_B receptors also exist on vascular smooth muscle cells of certain blood vessels of a variety of species including rats, pigs, dogs (Ihara *et al.*, 1991; Bigaud & Pelton, 1992; Teerlink *et al.*, 1994) and man (Davenport *et al.*, 1995), where they mediate vasoconstriction.

An increase in the serum levels of ET-1 has been documented in many cardiovascular disorders including circulatory shock. Pronounced rises in the serum levels of ET-1 occur in experimental endotoxaemia in rats (Sugiura *et al.*, 1989), dogs (Nakamura *et al.*, 1991), pigs (Pernow *et al.*, 1989) and sheep (Morel *et al.*, 1989). More importantly, enhanced ET-1 serum levels have also been documented in humans with sepsis and septic shock (Pittet *et al.*, 1991; Takakuwa *et al.*, 1994). In man, the serum levels of ET-1 correlate positively with the severity of endotoxaemia (Pittet *et al.*, 1991) and are lower in survivors than in non-survivors of septic shock (Takakuwa *et al.*, 1994).

The multiple organ dysfunction syndrome (MODS) in shock is defined as the dysfunction of a specific organ (e.g. lung, liver, kidney, etc) of a degree that homeostasis cannot be maintained without intervention. Most importantly, the progression of shock to MODS is associated with an increase in mortality from 25–30% (in the absence of MODS) to 90–100% (see Baue, 1993).

The question as to whether endogenous ET-1 contributes to the underlying pathophysiology of the MODS associated with septic shock and, hence, whether selective or non-selective endothelin receptor blockade is of benefit is still controversial. The non-selective ET_A/ET_B receptor antagonist SB 209670

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(Ohlstein *et al.*, 1994; Douglas *et al.*, 1995) aggravates the circulatory failure (Gardiner *et al.*, 1995; Ruetten *et al.*, 1996) as well as the renal and liver dysfunction caused by endotoxin in the rat (Ruetten *et al.*, 1996). Although the rapid release of endogenous ET-1 in endotoxaemia serves to maintain blood pressure and organ perfusion (beneficial effect of ET-1), excessive rises in the serum levels of ET-1 for longer periods are also associated with excessive vasoconstriction in some vascular beds (harmful effects of ET-1) (Ruetten *et al.*, 1996). In order to gain a better understanding of the receptors which mediate the above effects of endogenous ET-1 in endotoxaemia, we have now investigated the effects of the selective ET_A receptor antagonist, BQ-485 (Nakahashi *et al.*, 1995), and the selective ET_B receptor antagonist, BQ-788 (Ishikawa *et al.*, 1994), on (i) systemic haemodynamics, (ii) vascular hyporeactivity, (iii) renal function, (iv) liver function and integrity, and (v) acid-base balance in a rat model of endotoxic shock.

Methods

Measurement of haemodynamic changes

Male Wistar rats (240–320 g; Glaxo Laboratories Ltd., Greenford, Middlesex) were anaesthetized with thiopentone sodium (Intraval Sodium; 120 mg kg^{-1}, i.p.) and anaesthesia was maintained by supplementary injections of thiopentone sodium (approximately 1–2 mg kg^{-1} h^{-1}, i.v. as required). The trachea was cannulated to facilitate respiration and rectal temperature was maintained at 37°C with a homeothermic blanket (BioScience, Sheerness, Kent, U.K.). The right carotid artery was cannulated and connected to a pressure transducer (P23XL, Spectramed, Statham, Oxnard, CA, U.S.A.) for the measurement of phasic and mean arterial blood pressure (MAP) and heart rate (HR) which were displayed on a Grass model 7D polygraph recorder (Grass Instruments, Quincy, MA, U.S.A.). The femoral vein and jugular vein were cannulated for the administration of drugs. Upon completion of the surgical procedure, cardiovascular parameters were allowed to stabilize for 15 min.

After baseline haemodynamic parameters had been recorded animals were challenged with a submaximal (with respect to the pressor response) dose (Thiemermann *et al.*, 1993) of noradrenaline (NA, 1 μg kg^{-1}, i.v.). Different groups of animals received (5 min later) infusions of vehicle (saline, 0.6 ml kg^{-1} h^{-1}, $n=15$), or BQ-788 (10 nmol kg^{-1} min^{-1} in 0.6 ml kg^{-1} h^{-1} saline, $n=10$; Allcock *et al.*, 1995) or BQ-485 (10 nmol kg^{-1} min^{-1} in 0.6 ml kg^{-1} h^{-1} saline, $n=10$). Fifteen min after treatment with vehicle, BQ-788 or BQ-485, animals received either vehicle (saline, 1 ml kg^{-1}, i.v., $n=12$) or *E. coli* lipopolysaccharide (LPS, 10 mg kg^{-1}, i.v., $n=29$) as a slow injection over 10 min. The pressor response to NA was reassessed at every hour after LPS. All haemodynamic parameters were recorded for a further 360 min period.

Six hours after injection of LPS or vehicle, blood was taken to measure the changes in the serum levels of various biochemical marker enzymes of organ function and integrity (see below).

Quantification of renal and liver function and injury

Six hours after the injection of LPS, and immediately before the animals were killed with an overdose of anaesthetic, 1.5 ml of blood was collected from a catheter placed in the carotid artery. The blood sample was centrifuged (6,000 r.p.m. for 3 min; Biofuge 15, Heraeus, Cologne, Germany) to prepare serum. All serum samples were analysed within 24 h by a contract laboratory for veterinary clinical chemistry (Vetlab Services, Horsham, Sussex, U.K.). Liver function and integrity were assessed by measuring the rise in the serum levels of glutamate-pyruvate-transaminase (GPT, a specific marker for hepatic parenchymal injury); glutamate-oxalacetate-transaminase (GOT, a non-specific marker for hepatic parenchymal injury); bilirubin (a specific marker for the development of cholestasis, and, more importantly, a specific marker for the development of liver failure, see Baue, 1993). Renal function was assessed by measuring the rise in the serum levels of creatinine (an indicator of reduced glomerular filtration rate, and hence, renal failure) and urea (an indicator of impaired excretory function of the kidney and/or increased catabolism).

Evaluation of acid-base balance and blood gases

At time 0 and 15 min, 60 min, 180 min and 360 min after injection of LPS, 100 μl of blood was collected in glass tubes (Bilbate Ltd., Daventry, Northants, U.K.) from a catheter placed in the carotid artery for subsequent blood gas analysis. Blood gases were immediately measured by using a Corning 168 pH/Blood Gas Analyser (Corning Ltd, Halstead, Essex, U.K.). The blood gas analyser directly measures pH, $PaCO_2$ and PaO_2 and calculates bicarbonate (HCO_3^-).

Measurement of serum nitrite

Six hours after injection of LPS, 1 ml of blood was collected from the arterial catheter. The blood sample was centrifuged (15,000 r.p.m. for 3 min; Biofuge 15, Heraeus, Cologne, Germany) to prepare serum. The amount of nitrite, an indicator of NO formation, in the serum were measured by the Griess reaction (Green *et al.*, 1981; Gross *et al.*, 1990) by adding 100 μl of Griess reagent (1% sulphanilamide and 0.1% naphthylethylenediamide in 5% phosphoric acid) to 100 μl samples of serum. The optical density at 550 nm (OD_{550}) was measured by use of a Molecular Devices microplate reader (Anthos Labtec Instruments, Richmond, CA, U.S.A.). Nitrite concentrations were calculated by comparison with OD_{550} of a standard solution of sodium nitrite prepared in normal control serum.

Materials

Bacterial lipopolysaccharide (*E. coli* serotype 0.127:B8), trifluoroacetic acid, hydrochloric acid, sulphuric acid, sulphanilamide, naphthylethylenediamide, phosphoric acid and noradrenaline bitartate were obtained from Sigma Chemical Co. (Poole, Dorset, U.K.). Sodium thiopentone (Intraval Sodium) was obtained from Rhone Mérieux Ltd. (Harlow, Essex, U.K.). BQ-7882 and BQ-4853 were generous gifts from Dr M. Yano (Banyu Pharmaceutical Co., Ltd, Japan).

Statistical evaluation

All values in the figures and the text are expressed as mean ± s.e.mean of n observations, where n represents the number of animals or blood samples studied. A two-way analysis of variance (ANOVA) followed, if appropriate, by a Bonferoni's test was used to compare means between groups. Student's unpaired t test was used to compare means between groups. A P-value of less than 0.05 was considered to be statistically significant.

Results

Effects of the selective ET_A-receptor antagonist BQ-485 or the selective ET_B-receptor antagonist BQ-788 on the circulatory failure caused by endotoxaemia

Baseline values of mean arterial blood pressure (MAP) or heart rate were not significantly different between any of the experimental groups studied (Figure 1). In animals without endotoxaemia, infusion of vehicle ($n=5$), BQ-485 ($n=5$) or BQ-788 ($n=5$) did not result in any significant changes in MAP or heart rate (Table 1). Injection of LPS (10 mg kg^{-1}, i.v.) resulted in a rapid, but transient fall of MAP of 50 to 75 mmHg within 10 min ($P < 0.05$, $n=15$; Figure 1a). The

MAP values of rats treated with LPS remained above 95 mmHg from 60 to 240 min, and fell significantly towards the end of the experimental period at 360 min (Figure 1a). Treatment of LPS-rats with a continuous infusion of the selective ET_A-receptor antagonist BQ-485 (10 nmol kg^{-1} min^{-1}, commencing 15 min prior to LPS and continued throughout the experiment) significantly enhanced the delayed hypotension caused by endotoxin (Figure 1a). In contrast, continuous infusion of the selective ET_B-receptor antagonist BQ-788 (10 nmol kg^{-1} min^{-1}, commencing 15 min prior to LPS and continued throughout the experiment) significantly attenuated the delayed fall in MAP elicited by endotoxin (Figure 1a).

Endotoxaemia for 360 min also resulted in a small, time-dependent increase in heart rate (Figure 1b). This increase in heart rate caused by LPS was not affected by infusion of either BQ-485 or BQ-788 (Figure 1b).

Effects of the selective ET_A-receptor antagonist BQ-485 or the selective ET_B-receptor antagonist BQ-788 on the vascular hyporeactivity to noradrenaline caused by endotoxaemia

The mean baseline values for the pressor response to NA (1 μg kg^{-1}, i.v.) ranged from 35±4 to 42±5 mmHg and were not significantly different between any of the experimental groups studied. In animals without endotoxaemia, infusion of vehicle, BQ-485 or BQ-788 had no significant effect on the pressor responses elicited by NA (Table 1).

Endotoxaemia resulted in a rapid, biphasic attenuation of the pressor responses elicited by NA ($P < 0.05$, $n = 15$, Figure 2). This vascular hyporeactivity was unaffected in LPS-rats receiving an infusion of BQ-485 ($n = 7$, Figure 2). In contrast, infusion of BQ-788 significantly enhanced the pressor responses to NA between 120 and 300 min ($P < 0.05$, $n = 7$, Figure 2).

Effects of the selective ET_A-receptor antagonist BQ-485 or the selective ET_B-receptor antagonist BQ-788 on the renal and liver dysfunction caused by endotoxaemia

Endotoxaemia for 360 min resulted in rises in the serum levels of urea and creatinine. Treatment of LPS-rats with either BQ-788 (10 nmol kg^{-1} min^{-1}, $n = 7$) or BQ-485 (10 nmol kg^{-1} min^{-1}, $n = 7$) had no effect on the rises in the serum levels of urea and creatinine (Figure 3). Six hours of endotoxaemia

Table 1 Effects of vehicle (saline, $n = 6$), BQ-485 (10 nmol kg^{-1}, $n = 3$) or BQ-788 (10 nmol kg^{-1} min^{-1}, $n = 3$) on mean arterial blood pressure (MAP), heart rate (HR) and the pressor responses to noradrenaline (NA) in rats treated with saline rather than LPS

	Time (min)			
Group	*0*	*120*	*240*	*360*
Saline				
MAP (mmHg)	123±2	124±2	117±3	114±3
HR (beats min^{-1})	432±6	420±7	418±8	405±5
NA (mmHg)	35±4	41±5	43±3	43±3
BQ-485				
MAP (mmHg)	119±4	118±4	116±3	114±4
HR (beats min^{-1})	418±13	408±12	416±9	413±6
NA (mmHg)	42±5	45±3	38±5	40±5
BQ-788				
MAP (mmHg)	125±4	121±5	119±4	116±5
HR (beats min^{-1})	411±15	409±12	417±8	402±8
NA (mmHg)	37±6	39±4	41±5	42±5

Note that in rats which did not receive *E. Coli* lipopolysaccharide (LPS), infusion of saline, BQ-485 or BQ-788 did not cause a significant change in MAP, HR or the pressor responses elicited by NA over time.

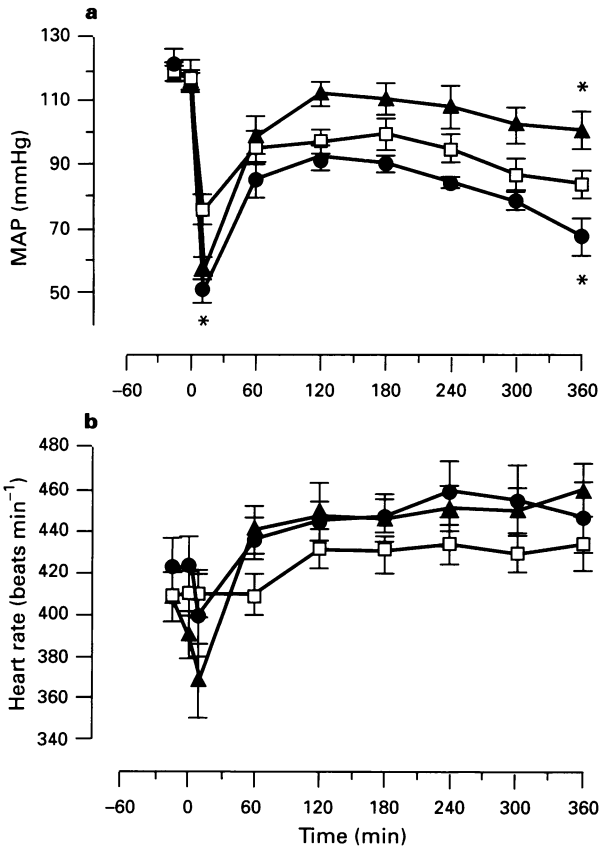

Figure 1 Effects of the selective ET_A-receptor antagonist BQ-485 or the selective ET_B-receptor antagonist BQ-788 on the circulatory failure in endotoxic shock in the anaesthetized rat. Depicted are the changes in (a) mean arterial blood pressure (MAP) and (b) heart rate in rats which had received *E. coli* lipopolysaccharide (LPS; 10 mg kg^{-1}, i.v.) and were pretreated with either vehicle (saline, 0.6 ml kg^{-1} h^{-1}, □, $n = 15$), BQ-788 (10 nmol kg^{-1} min^{-1}, ▲, $n = 7$) or BQ-485 (10 nmol kg^{-1} min^{-1}, ●, $n = 7$). Data are expressed as mean±s.e. mean (vertical lines) of n observations. *$P < 0.05$ represents significant differences when compared to LPS-control at the same time point.

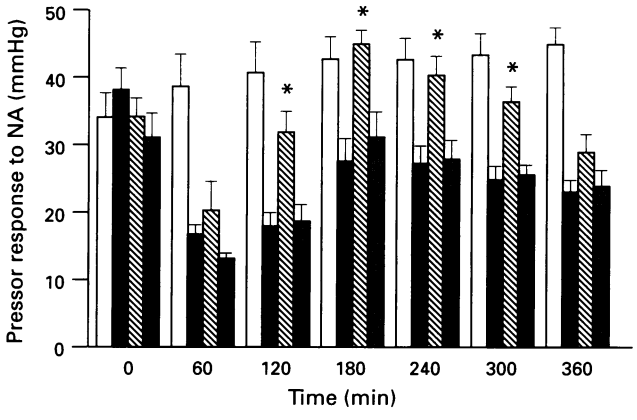

Figure 2 Effects of the selective ET_A-receptor antagonist BQ-485 and the selective ET_B-receptor antagonist BQ-788 on the pressor responses to noradrenaline (NA) in endotoxic shock in the anaesthetized rat. These pressor responses to noradrenaline were measured in rats which had received vehicle (saline) for *E. coli* lipopolysaccharide (LPS) (open columns, $n = 6$), LPS (10 mg kg^{-1}, i.v.) for 360 min and were treated with either vehicle (saline, 0.6 ml kg^{-1} h^{-1}, solid columns, $n = 15$), BQ-788 (10 nmol kg^{-1} min^{-1}, hatched columns, $n = 7$) or BQ-485 (10 nmol kg^{-1} min^{-1}, stippled columns, $n = 7$). Data are expressed as mean±s.e.mean (vertical lines) of n observations. *$P < 0.05$ represents significant differences when compared to LPS-controls at the same time point.

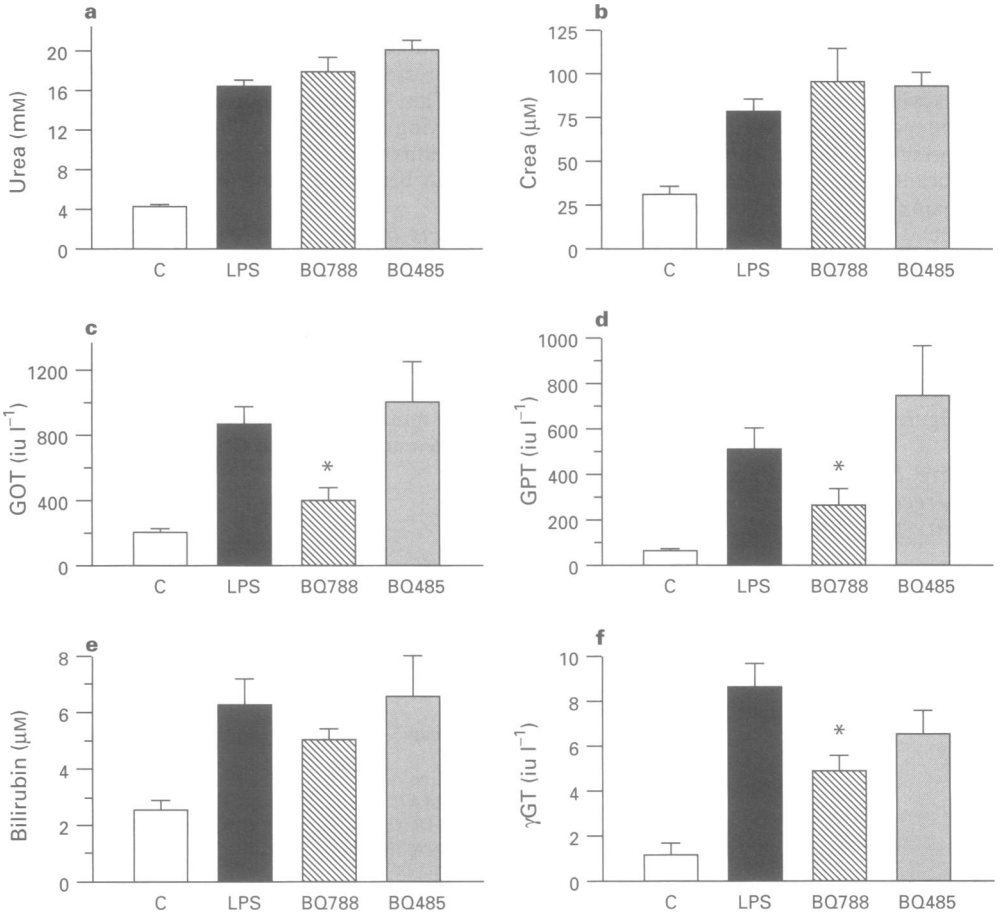

Figure 3 Effects of the selective ET_A-receptor antagonist BQ-485 and the selective ET_B-receptor antagonist BQ-788 on the *E. coli* lipopolysaccharide (LPS)-induced increases in the serum concentrations of (a) urea, (b) creatinine, (c) glutamate-oxalate-transferase (GOT), (d) glutamate-pyruvate-transferase (GPT), (e) bilirubin and (f) γ-glutamyl transferase (γGT). These enzymes activities were measured in the serum obtained from rats that had received vehicle (saline) for LPS (open columns, $n=6$) or *E. coli* lipopolysaccharide (10 mg kg^{-1}, i.v.) for 360 min and were treated with either vehicle (saline, 0.6 ml kg^{-1} h^{-1}, solid columns, $n=15$), BQ-788 (10 nmol kg^{-1} min^{-1}, hatched columns, $n=7$) or BQ-485 (10 nmol kg^{-1} min^{-1}, stippled columns, $n=7$). Data are expressed as mean ± s.e.mean (vertical lines) of n observations. $^*P<0.05$ represents significant differences when compared to LPS-controls.

was also associated with significant increases in the serum levels of GOT, GPT, bilirubin and γGT. Treatment of LPS-rats with BQ-485 had no effect on the rises in GOT, GPT, γGT and bilirubin. In contrast, treatment of LPS-rats with BQ-788 largely attenuated the rise in the serum levels of GPT, GOT and γGT, but had no significant effect on the rise in bilirubin (Figure 3). Endotoxaemia also caused a pronounced rise in the serum levels of nitrite, which was not affected by pretreatment of LPS-rats with either BQ-485 or BQ-788 (Figure 4).

In animals without endotoxaemia, infusion of vehicle ($n=5$), BQ-485 ($n=5$) or BQ-788 ($n=5$) did not result in any significant changes in the serum levels of creatinine, urea, GOT, GPT, bilirubin, γGT or nitrite (Table 2).

Effects of the selective ET_A-receptor antagonist BQ-485 or the selective ET_B-receptor antagonist BQ-788 on acid-base balance and blood gases in endotoxaemia

Injection of LPS (10 mg kg^{-1}, i.v.) caused within 15 min an acute metabolic acidosis as indicated by the falls in pH and HCO_3^- (Figure 5). The fall in pH, but not the fall in HCO_3^- returned to baseline within 180 min. In addition, endotoxaemia for 360 min caused a progressive fall in $PaCO_2$ (Figure 4). Pretreatment of LPS-rats with either BQ-485 or BQ-788 had no effects on pH, $PaCO_2$, PaO_2 and HCO_3^- (Figure 5). Please note that no significant alteration in any of the above parameters was observed in rats without endotoxaemia treated with either vehicle, BQ-485 or BQ-788 (Table 2).

Discussion

This study demonstrates that the selective ET_B receptor antagonist BQ-788 attenuates the (i) circulatory failure (delayed hypotension, vascular hyporeactivity to noradrenaline), and (ii) the rise in the serum levels of GOT, GPT and γGT (liver dysfunction and injury) caused by endotoxin in the anaesthetized rat. In contrast, BQ-788 did not affect the rise in the serum levels of urea and creatinine (acute renal failure) or the alterations in acid base balance caused by endotoxic shock.

The rises in the serum levels of these biochemical markers are likely to reflect progressive organ dysfunction, failure or injury. Indeed, a greater than 2 fold rise in the serum levels of GOT or GPT is indicative of the development of liver cell injury, while rises in the serum levels of bilirubin and γGT suggest liver dysfunction (see Baue, 1993). This study demonstrated that endotoxaemia for 360 min in the anaesthetized rat results in significant rises in the serum levels of GOT (4 fold), GPT (5 fold), bilirubin (2 fold) and γGT (7 fold) suggesting the development of liver cell injury and dysfunction. Treatment of LPS-rats with BQ-788 attenuated the rises in the serum levels of GOT, GPT and γGT and, to a lesser extent, of bilirubin. Thus, BQ-788 reduced the degree of liver cell injury and dysfunction caused by endotoxin in the rat. In contrast, treatment of LPS-rats with the selective ET_A receptor antagonist BQ-485 had no effects on the hepatocellular dysfunction and injury caused by endotoxin.

What, then, is the mechanism(s) by which BQ-788 prevents

liver dysfunction and injury in rats with endotoxic shock? The organ injury associated with shock is due to an impairment in tissue oxygen extraction and ultimately tissue hypoxia (resulting in metabolic acidosis). Tissue oxygen extraction in shock is dependent on regional organ blood flow distribution and, most importantly, oxygen delivery ('supply-dependency' of tissue oxygenation) and, therefore, blood pressure (perfusion-pressure) and cardiac output. Indeed, endotoxic shock results in a maldistribution of hepatic blood flow as well as a fall in hepatic artery perfusion pressure secondary to hypotension and myocardial dysfunction (see Bankey & Cerra, 1993). We propose that the prevention by BQ-788 of the ob-

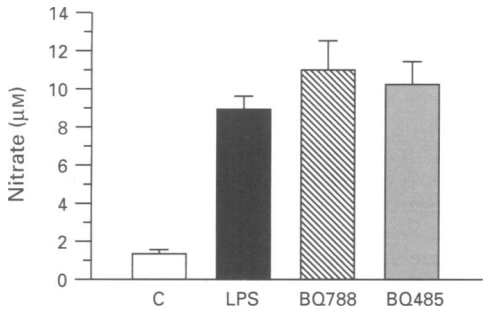

Figure 4 Effects of the selective ET_A-receptor antagonist BQ-485 and the selective ET_B-receptor antagonist BQ-788 on the *E. Coli* lipopolysaccharide (LPS)-induced increase in serum nitrite. Nitrite was measured in serum samples obtained at 360 min from rats that had received vehicle (saline) for LPS (open columns, $n=6$), or *E. coli* lipopolysaccharide (10 mg kg^{-1}, i.v.) for 360 min and were treated with either vehicle (saline, 0.6 ml kg^{-1} h^{-1}, solid columns, $n=15$), BQ-788 (10 nmol kg^{-1} min^{-1}, hatched columns, $n=7$) or BQ-485 (10 nmol kg^{-1} min^{-1}, stippled columns, $n=7$). Data are expressed as mean ± s.e.mean (vertical lines) of n observations.

Table 2 Effects of vehicle (saline, $n=6$), BQ-485 (10 nmol kg^{-1} min^{-1}, $n=3$) or BQ-788 (10 nmol kg^{-1} min^{-1}, $n=3$) on indicators of liver and renal injury as well as acid base balance and serum nitrite in rats treated with saline rather than LPS

	Experimental group		
Parameter	*Saline*	*BQ-485*	*BQ-788*
Urea (mmol l^{-1})	4.2±0.2	4.5±0.4	4.8±0.5
Creatinine (μmol l^{-1})	31±4	35±4	37±5
GOT (iu l^{-1})	202±18	189±28	243±36
GPT (iu l^{-1})	64±4	78±6	59±5
Bilirubin (μmol l^{-1})	2.6±0.3	2.3±1.3	1.9±0.8
γGT (iu l^{-1})	1.7±0.3	2.1±0.5	2.4±0.8
Nitrite (μM)	1.8±0.2	1.8±0.5	2.4±0.6
PaO_2 (mmHg)	86±5	79±7	81±6
$PaCO_2$ (mmHg)	42±3	39±4	41±5
pH	7.38±0.02	7.41±0.08	7.35±0.06
HCO_3	26.0±1.1	24.8±1.5	23.3±1.9

The above parameters were measured at 360 min after injection of the vehicle for *E. Coli* lipopolysaccharide (LPS); For key to abbreviations used see text.

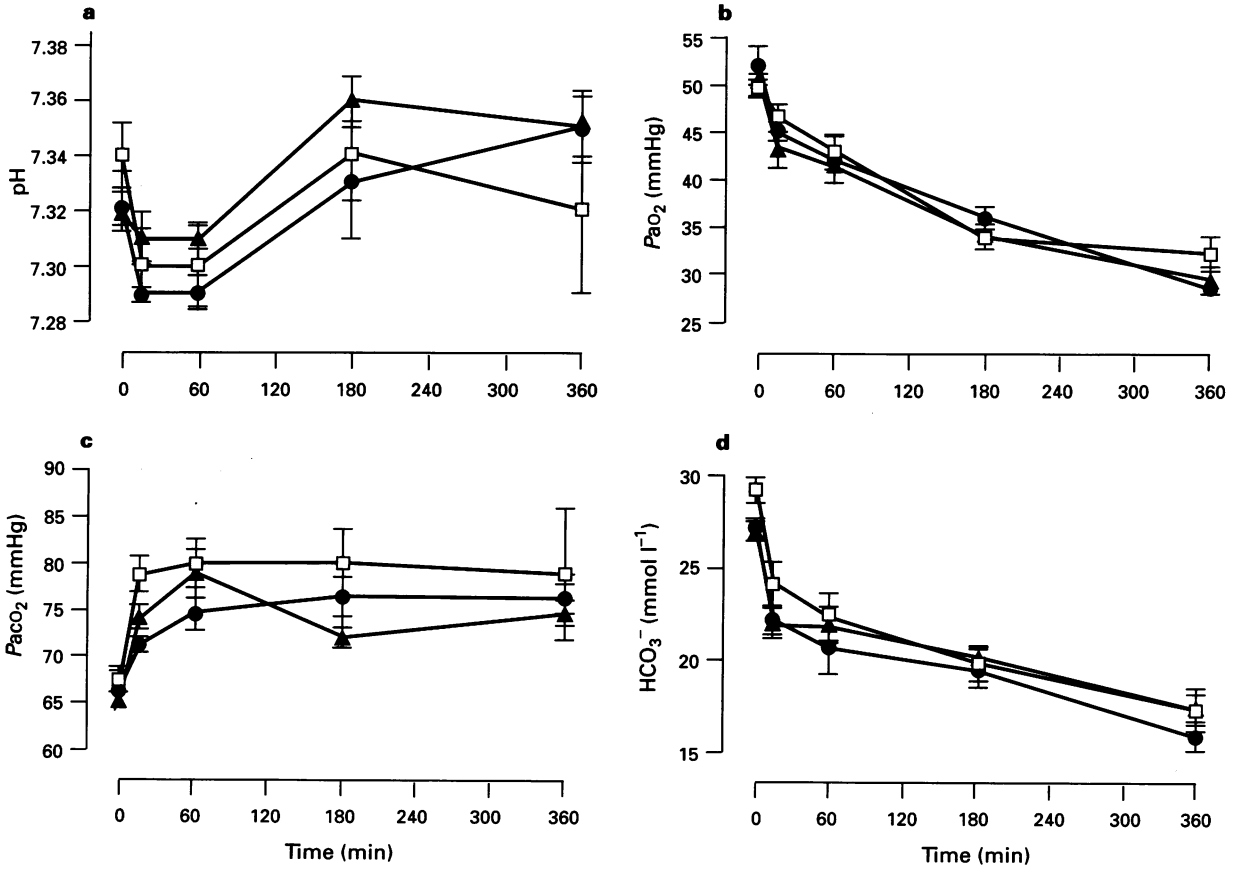

Figure 5 Effects of the selective ET_A-receptor antagonist BQ-485 or the selective ET_B-receptor antagonist BQ-788 on the (a) pH, (b) arterial oxygen tension (Pao_2), (c) arterial carbon dioxide tension ($Paco_2$) and (d) standard bicarbonate (HCO_3^-) were measured in arterial blood samples obtained from rats that had received *E. coli* lipopolysaccharide (LPS, 10 mg kg^{-1}, i.v.) for 360 min. LPS-rats were treated with either vehicle (saline, 0.6 ml kg^{-1} h^{-1}, □, $n=15$), BQ-788 (10 nmol kg^{-1} min^{-1}, ▲, $n=7$) or BQ-485 (10 nmol kg^{-1} min^{-1}, ●, $n=7$). Data are expressed as mean ± s.e.mean (vertical lines) of n observations.

served liver dysfunction and injury is due to an improvement in hepatic blood flow (and oxygen delivery) as a result of inhibition of sinusoidal constriction, inhibition of pre-sinusoidal contraction, and/or augmentation of blood and perfusion pressure.

Inhibition of sinusoidal contraction

ET-1 causes the constriction of hepatic sinusoids (Okumura *et al.*, 1994; Zhang *et al.*, 1994). However, due to the absence of vascular smooth muscle cells within the sinuoid, other cell types must be implicated in the regulation of sinusoidal diameter by ET-1 (or other mediators). Hepatic stellate cells (Ito cells), which are perisinusoidal cells, exhibit features of smooth muscle cells and contribute to the regulation of sinusoidal blood flow (Ramadori *et al.*, 1990; Ramadori, 1991; Sakamato, 1991; Rockey *et al.*, 1993). Like smooth muscle cells, Ito cells also express both ET_A and ET_B receptors (Housset *et al.*, 1993), activation of which leads to Ito cell contraction (Sakamoto, 1991). Activation of Ito cells (e.g. by endotoxin or cytokines) leads to the expression markers of smooth muscle cells (Ramadori *et al.*, 1990; Rockey *et al.*, 1993) and expression of the mRNAs for ET-1 as well as for ET_A and ET_B receptors (Housset *et al.*, 1993). The specific ET-receptor mediating Ito cell contraction varies between species. In the rat, activation of ET_B-receptors (Rockey, 1995) and/or ET_A-receptors (Zhang *et al.*, 1995) mediates the contraction of Ito cells caused by ET-1. However, in activated human Ito cells activation of ET_A-receptors accounts for the increase in intracellular calcium, cell contraction and mitogenicity caused by ET-1 (Pinzani *et al.*, 1996). In addition to Ito cells, sinusoid constriction may also be caused by endothelial cells or Kupffer cells, both of which express only ET_B receptors (Housset *et al.*, 1993). Indeed, endotoxaemia causes the swelling of Kupffer cells resulting in the obstruction of the sinusoidal lumen (McCluskey & Reilly, 1993).

Inhibition of pre-sinusoidal contractions

Activation of the ET_B-receptors (but not of ET_A receptors) in the pre-sinusoidal vasculature, e.g. in portal venules, results in vasoconstriction and, hence, may also contribute to a reduction in hepatic blood flow (Kurihara *et al.*, 1992; Bauer *et al.*, 1994; Zhang *et al.*, 1995). As endotoxaemia (this model) results in a rapid and substantial increase in the plasma levels of ET-1 (Ruetten *et al.*, 1996), any reduction in sinusoidal blood flow is likely to be due to extra-sinusoidal as well as sinusoidal constriction.

Improvement in perfusion pressure

Selective blockade of ET_B receptors with BQ-788 attenuated the delayed hypotension caused by endotoxin and, therefore, should result in an augmentation of perfusion pressure (hepatic artery) to the liver. The mechanism by which BQ-788 prevented the delayed fall in blood pressure is largely unclear. The delayed circulatory failure (hypotension and vascular hyporeactivity to vasoconstrictor agents) caused by endotoxin in the anaesthetized rat is due to an enhanced formation of NO by the inducible isoform of NO synthase (iNOS; Szabo *et al.*, 1993; Thiemermann *et al.*, 1995). BQ-788 did not attenuate the endotoxin-induced rise in serum nitrite, a reliable indicator of the degree of iNOS induction in organs and vessels in this model (De Kimpe *et al.*, 1995; Thiemermann *et al.*, 1995). Interestingly, ET_B receptors are responsible for the clearance of ET-1 from the circulation and BQ-788 enhances the vasoconstrictor responses elicited by exogenous ET-1 (Fukuroda *et al.*, 1994). The release of endogenous ET-1 serves to maintain blood pressure and organ perfusion in conscious (Gardiner *et al.*, 1995) and anaesthetized rats with endotoxaemia (Ruetten *et al.*, 1996). Prevention by BQ-788 of ET-1 clearance and, hence, an an increase in the plasma levels of ET-1 may well explain the improvement in blood pressure caused by BQ-788. Attenuation by BQ-788 of the vascular hyporeactivity to noradrenaline may also contribute to the beneficial haemodynamic effects of the ET_B-receptor antagonist in rats with endotoxic shock.

The criteria for the definition of organ failure in sepsis (see Baue, 1993 for review) also state that a 2 fold increase in serum creatinine (regardless of polyuria or anuria) indicates acute renal failure. We demonstrate here that 360 min of endotoxaemia are associated with more than 2 fold rises in the serum levels of creatinine and urea and, hence, acute renal failure. Although endogenous ET-1 has been implicated in the pathophysiology of the acute renal injury associated with endotoxaemia (see Warner, 1993; Miller & Thiemermann, 1996), we found that neither BQ-788 nor BQ-485 attenuated the rise in the serum levels of urea and creatinine caused by endotoxin in the rat. Thus, this study provides no evidence for a role for ET-1 in the pathogenesis of the endotoxin-induced acute renal failure in the rat. Endotoxaemia also resulted (within 15 min) in falls in pH, HCO_3^- and base excess and, hence, a metabolic acidosis. The continuous fall in $PaCO_2$ observed during the remainder of the experiment was associated with a normalisation of pH suggesting that the acute lactate acidosis was largely compensated by an increase in respiration rate (increase in PaO_2, fall in $PaCO_2$ and HCO_3^-). This metabolic acidosis was, however, not significantly affected by treatment of LPS-rats with either BQ-788 or BQ-485.

In conclusion, this study demonstrates that the ET_B receptor antagonist BQ-788, but not the ET_A receptor antagonist BQ-485, reduces the circulatory failure (hypotension, vascular hyporeactivity to noradrenaline) and largely attenuates the degree of liver dysfunction and hepatocellular injury caused by endotoxaemia in the rat. Our study does not provide direct evidence for the mechanism by which BQ-788 prevents liver dysfunction and injury in rats with endotoxaemia. As there is convincing evidence that activation of ET_B-receptors in the rat leads to contraction of portal venules and Ito cells, we hypothesise that the observed effects of BQ-788 on liver function/integrity are due to an improvement in hepatic blood flow secondary to inhibition of pre-sinusoidal constriction, inhibition of sinusoidal constriction, and (to a lesser degree) improvement in perfusion pressure. Although one could also argue that the observed liver dysfunction is (at least in part) due to a direct effect of ET-1 on hepatocytes, this is extremely unlikely, for hepatocytes do not express mRNA for either ET_A or ET_B receptors (Housset *et al.*, 1993).

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