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European Journal of Pharmacology 386 (1999) 235–245

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Effects on haemodynamics by selective endothelin ET_B receptor and combined endothelin ET_A/ET_B receptor antagonism during endotoxin shock

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Received 26 April 1999; received in revised form 27 August 1999; accepted 29 October 1999

Abstract

The endothelin system is highly activated during endotoxin and septic shock. To investigate this matter the selective non-peptide endothelin ET_B receptor antagonist A-192621 ([2*R*-(2α,3β,4α)]-4-(1,3-benzodioxol-5-yl)-1-[2-[2,6-diethylphenyl)amino]-2-oxoethyl]-2-(4-propoxy-phenyl)-3-pyrrolidinecarboxylic acid) was administered alone and in combination with the selective non-peptide endothelin ET_A receptor antagonist PD 155080 (sodium 2-benzo[1,3]dioxol-5-yl-3-benzyl-4-(4-methoxy-phenyl)-4-oxobut-2-enoate) during established porcine endotoxin shock. Cardiopulmonary vascular function, metabolic parameters and plasma endothelin-1-like immunoreactivity levels were compared to a control group only receiving endotoxin. Administration of A-192621 alone resulted in cardiovascular collapse and death whereas combining A-192621 with PD 155080 abolished endotoxin induced pulmonary hypertension, enhanced cardiac performance and improved systemic oxygen delivery and acid–base balance. The beneficial effects of mixed endothelin ET_A/ET_B receptor antagonisms on the pulmonary and cardiovascular systems may result from blockage of constrictive endothelin receptors in and pulmonary circulation, reduced afterload and a direct inotropic effect. Possible mechanisms for the devastating effects by selective endothelin ET_B receptor antagonism include increased endothelin ET_A receptor-mediated vasoconstriction due to lack of endothelin ET_B receptormediated vasodilation and decreased endothelin clearance from endothelin ET_B receptor blockade. In conclusion, selective endothelin ET_B receptor antagonism is deleterious whereas combined endothelin ET_A and ET_B receptor antagonism has favourable effects on haemodynamics, suggesting participation of the endothelin system in cardiopulmonary dysfunction during endotoxin shock.

Keywords: Endothelin-1; Endothelin ET_A-receptor; Endothelin ET_B-receptor; A-192621; PD 155080; Endotoxaemia; Septic shock

1. Introduction

Endothelium-derived vasoactive substances have been shown to be of importance both under normal and various pathological conditions (Bassenge, 1996). One of these mediators, endothelin-1, with potent vasoconstrictive properties has been suggested to be involved in several diseases such as heart failure (Kiowski et al., 1995), ischaemia/reperfusion injury (Pernow and Wang, 1997), subarachnoid haemorrhage (Ehrenreich and Schilling, 1995), primary pulmonary hypertension (Goldie et al., 1996) and sepsis (Battistini et al., 1996). Apart from vasoconstriction, endothelin-1 also may mediate mitogenic stimulation of smooth muscle cells (Piero, 1995), neutrophil activation (Caramelo et al., 1997) and lymph vessel constriction (Reeder and Ferguson, 1996). The effects of this 21 amino acid peptide, converted from its pro-form, big endothelin-1, by an isoenzyme family named endothelin converting enzyme, are mediated by at least two receptors, the endothelin ET_A and endothelin ET_B receptors. These two receptors are found in most mammals and through the development of different receptor antagonists and agonists, their functions have been shown to be more complicated than first thought. Both endothelin ET_A and endothelin ET_B (ET_{B2}) receptors located on smooth muscle cells mediate contraction whereas stimulation of a subgroup of endothelin ET_B receptors (ET_{B1}), located on the endothelium, cause va-

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0014-2999/99/$ - see front matter
PII: S0014-2999(99)00774-8

sodilation through release of prostacyclin and nitric oxide (NO) (De Nucci et al., 1988). Furthermore, the endothelin ET_{B1} receptors could be further subdivided depending on differences in sensitivity to various types of agonists and antagonists (MacLean et al., 1998; Miasiro et al., 1998). In addition, the endothelial endothelin ET_{B1} receptor seems to participate in the clearance of circulating endothelin-1 (Dupuis et al., 1996), a process that has been suggested to be mediated by a splice variant of the endothelin ET_B receptor (Elshourbagy et al., 1996). Other mechanisms proposed to be mediated by the endothelin ET_B receptor includes positive inotropy (Beyer et al., 1995) and macrophage induced inflammatory response (Sakurai-Yamashita et al., 1997). Complicating the issue even more is the suggested cross-talk between the endothelin ET_A and endothelin ET_B receptors, leading to increased endothelin ET_B receptor sensitivity during endothelin ET_A receptor antagonism (Ozaki et al., 1997). Therefore, the role of the different endothelin receptor subtypes in response to endothelin stimulation is still not fully elucidated.

Septic shock, a condition where endothelium-derived substances most likely play an important role, is often accompanied by cardiac dysfunction, pulmonary hypertension, systemic hypotension and impaired oxygen utilisation with systemic acidosis (Vincent, 1998). Experimental endotoxaemia shows a similar pattern (Parillo et al., 1990) and, like sepsis, is associated with an increase in circulating plasma endothelin-1 levels (Weitzberg et al., 1991; Shindo et al., 1998). Furthermore, cardiac dysfunction (Pittet et al., 1991) and acute respiratory distress syndrome (Druml et al., 1993), known features of septic shock, correlate to increased plasma endothelin-1 levels. Our group has previously shown that the mixed endothelin receptor antagonist, bosentan, has favourable effects on cardiopulmonary circulation both when administered before and during established porcine endotoxin shock (Weitzberg et al., 1996; Wanecek et al., 1997a). Interestingly, the selective non-peptide endothelin ET_A receptor antagonist PD 155080 also counteracts endotoxin-induced pulmonary hypertension but does not improve cardiac performance in the same pig model (Wanecek et al., 1999). Therefore, the aim of the present study was to investigate the involvement of the endothelin$_B$ receptor as well as to evaluate the mutual effects of the endothelin ET_A and endothelin ET_B receptors in the cardiopulmonary pathophysiology of porcine endotoxin shock. For this purpose, the selective non-peptide endothelin ET_B receptor antagonist A-192621 ([2*R*-(2α,3β,4α)]-4-(1,3-benzodioxol-5-yl)-1-[2-[2,6-diethylphenyl)amino]-2-oxoethyl]-2-(4-propoxyphenyl)-3-pyrrolidinecarboxylic acid) (Abbot Laboratories, IL, USA) was administered during established shock. To achieve a combined endothelin receptor blockade, A-192621 was also given in combination with PD 155080 (sodium 2-benzo[1,3]dioxol-5-yl-3-benzyl-4-(4-methoxyphenyl)-4-oxobut-2-enoate) (Parke Davis, NJ, USA). Cardiopulmonary vascular function was followed as well as metabolic parameters and plasma endothelin-1-like immunoreactivity levels. Furthermore, the two antagonists were also characterised, both in vivo and in vitro.

2. Materials and methods

The experimental protocol for this study was approved by the Ethics Committee for experimental animal research at the Karolinska Institute, Stockholm, Sweden.

2.1. Anaesthesia and surgical preparation

Eighteen pigs of both sexes (wt 17.3–23.4 kg) were fasted overnight with free access to water. After premedication with an i.m. injection of ketamine 20 mg kg^{-1} and atropine 25 μg kg^{-1} the animals were brought from the animal department. General anaesthesia was induced by an i.v. bolus injection of pentobarbital, 12 mg kg^{-1} and fentanyl, 10 μg kg^{-1} and maintained by a continuous infusion of pentobarbital, 6 mg kg^{-1} h^{-1} and fentanyl 5 μg kg^{-1} h^{-1}. After the reaction to pain from fore-hoof stimulation was extinguished, muscle paralysis, to prevent shivering, was achieved by an infusion of pancuronium bromide 0.1 mg kg^{-1} h^{-1}. The animals were tracheostomised and mechanically normoventilated with a gas mixture of oxygen and air ($FiO_2 = 0.30$) (Servo 900 ventilator, Siemens Elema, Sweden) with a respiratory frequency of 18 min^{-1}. The animals received a continuous infusion of isotonic saline with glucose 25 mg ml^{-1} at a rate of 20 ml kg^{-1} h^{-1} throughout the experiment. Body temperature was monitored by a rectal thermistor and maintained around 38–39°C by a heating pad. Left femoral vein and artery catheters were inserted for infusions and measurement of arterial blood pressure, respectively. A balloon-tipped pulmonary artery catheter was inserted through the right femoral vein to position in the pulmonary artery by pressure guidance. A catheter was placed in the urinary bladder by a small cystotomy. Finally the animals were placed in left position and the pentobarbital infusion rate was reduced to 3 mg kg^{-1} h^{-1}.

2.2. Haemodynamic measurements and blood gases

Arterial and pulmonary artery catheters were connected to pressure transducers and heart rate, mean arterial blood pressure, mean pulmonary artery pressure and central venous pressure were recorded continuously, while pulmonary capillary wedge pressure was registered intermittently on a polygraph (Grass 7B, Quincy, MA, USA). Cardiac index (indexed to body weight) was measured by thermodilution (Edwards Lab 9520A, St. Ana, CA, USA) and determined as the mean of a triplicate of 10 ml of ice-cold saline injections. The systemic vascular resistance index was calculated as: mean arterial blood pressure − central venous pressure/cardiac index and pulmonary vascular resistance index as: mean pulmonary artery pressure − pulmonary capillary wedge pressure/cardiac index. Left

ventricular stroke work index was calculated as: mean arterial blood pressure − pulmonary capillary wedge pressure × stroke volume index × 0.0136 and right ventricular stroke work index as: mean pulmonary artery pressure − central venous pressure × stroke volume index × 0.0136. Blood was collected from the arterial and pulmonary artery catheters for analysis of blood gases on an ILS 1610 blood gas analyser (Instrumental laboratories, USA). Systemic oxygen delivery index was calculated as: arterial oxygen saturation (SaO_2) × haemoglobin concentration × 0.0139 × cardiac index and systemic oxygen consumption index as: SaO_2 − mixed venous oxygen saturation (SvO_2) × haemoglobin concentration × 0.0139 × cardiac index. For estimation of pulmonary shunt fraction, the shunt equation, (((haemoglobin concentration × 1.39) + ((28.5 − (Arterial carbon dioxide partial pressure (PCO_2)/0.8)) × 0.225)) − ((haemoglobin concentration × SaO_2 × 0.0139) + (Arterial oxygen partial pressure (PaO_2) × 0.225)))/(((haemoglobin concentration · 1.39) + ((28.8 − $PaCO_2$/0.8)) × 0.225)) − ((haemoglobin concentration · SvO_2 · 0.0139) + (pulmonary artery oxygen partial pressure × 0.225))), was used.

2.3. Biochemical analysis

Plasma levels of endothelin-1-like immunoreactivity were analysed in arterial and pulmonary arterial plasma with radioimmunoassay as previously described (Hemsen, 1991). Haemoglobin concentration was measured spectrophotometrically (Haemoglobin photometer, LEO, Helsingborg, Sweden) and erythrocyte volume fraction was measured by means of a centrifuge (Microspin, Bayer, Gothenburg, Sweden). Pulmonary release or uptake of endothelin-1 was calculated as: pulmonary artery endothelin-1-like immunoreactivity − arterial endothelin-1-like immunoreactivity × cardiac output × (1-erythrocyte volume fraction /100). A negative value indicates a release from the pulmonary circulation. For arterial lactate measurements, an Accusport blood lactate system was used (Boeringer Mannheim Scandinavia, Stockholm, Sweden).

2.4. Endotoxin

Escherichia coli lipopolysaccharide endotoxin (serotype 0111:B4, Sigma, St. Louis, USA) was used. Prior to infusion, the endotoxin was dissolved in saline and heated in order to dissolve any precipitate.

2.5. Experimental protocol

After surgical preparation, the animals were allowed to stabilise for 1 h after which baseline measurements were made prior to onset of endotoxin challenge ($T_{1\,h}$ and $T_{0\,h}$). An i.v. endotoxin infusion was started with a rate of 2.5 μg kg^{-1} h^{-1} and was increased stepwise until reaching 20 μg kg^{-1} h^{-1} after 30 min. At $T_{2\,h}$ six animals received an i.v. bolus injection of A-192621 10 mg kg^{-1} (16.5 μmol kg^{-1}), followed by a continuous i.v. infusion of 5 mg kg^{-1} h^{-1}(8.2 μmol kg^{-1} h^{-1}), maintained throughout the experiment ($T_{5\,h}$). A-192621 was dissolved in 2% dimethylsulfoxide (DMSO) and a total amount of 100 ml was administered. Another group of six animals received A-192621 as described above in combination with PD 155080, administered as a bolus of 10 mg kg^{-1} (22.8 μmol kg^{-1}) during 30 min, followed by an i.v.-continuous infusion of 5 mg kg^{-1}·h^{-1} (11.4 μmol kg^{-1} h^{-1}). PD 155080 was dissolved in saline to a total amount of 100 ml. In this group, the saline–glucose infusion was reduced by the volume corresponding to the saline vehicle for PD 155080. Six animals receiving only endotoxin and 100 ml of DMSO vehicle served as controls. The endotoxin infusion was discontinued after 3 h ($T_{3\,h}$) in all groups.

Mean arterial blood pressure, heart rate and mean pulmonary artery pressure were continuously followed. Every 30 min cardiac output, pulmonary capillary wedge pressure and central venous pressure were recorded and cardiac index, systemic vascular resistance index, pulmonary vascular resistance index, left ventricular stroke work index and right ventricular stroke work index were calculated. Blood samples were drawn hourly for analysis of haemoglobin concentration, erythrocyte volume fraction, blood gases, arterial lactate and endothelin-1-like immunoreactivity. At $T_{5\,h}$ the experiments were terminated and the

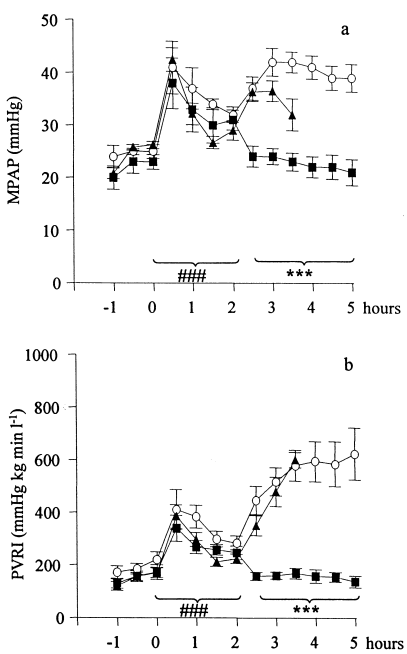

Fig. 1. Mean pulmonary artery pressure (MPAP) (a) and pulmonary vascular resistance index (PVRI) (b) during endotoxin infusion, started at 0h. At 2h one group ($n = 6$) (▲) received an intravenous bolus of A-192621 (10 mg kg^{-1}) followed by a continuous infusion (5 mg kg^{-1} h^{-1}). Another group ($n = 6$) (■) received A-192621 in the same fashion in combination with a bolus of PD 155080 (10 mg kg^{-1}) followed by a continuous infusion (5 mg kg^{-1} h^{-1}). Control pigs ($n = 6$) (○) only received endotoxin infusion and vehicle. $^{###}P < 0.001$ for differences in time before intervention, $^{***}P < 0.001$ for differences between groups after intervention.

animals were sacrificed by a lethal dose of pentobarbital injected into a central vein.

2.6. Receptor binding studies

One pig was anaesthetised and sacrificed as described above, whereupon biopsies were obtained from the apex of the heart and placed in cold saline. The tissue was homogenised using a polytron (Kinematica, Luzern, Switzerland) and then centrifuged for 10 min at $1000 \times g$ after which the supernatants were decanted and further centrifuged for 30 min at $10\,000 \times g$. The second supernatants were discarded and the remaining pellets, containing the membranes, were resuspended in 0.9% NaCl, pH 7.4, containing 5 mmol l^{-1} HEPES. The protein concentrations were measured using BCA Protein Assay Kit Reagent (Pierce, Rockford, IL, USA). The assay buffer contained in mmol l^{-1}: NaCl, 137; KCl, 2.68; $MgCl_2$, 2.05; $CaCl_2$, 1.80; and HEPES, 20; pH 7.4. Before use 0.1% bovine serum albumin was added. The kinetic studies were performed on diluted membranes (50–150 μg protein) to which radioligand, 1.5 nmol l^{-1} ^{125}I-labelled IRL 1620 ([*N*-succinyl-[Glu^{9},Ala^{11,15}]-ET-1(8–21)]), a selective endothelin ET_B receptor agonist (DuPont NEN, MA, USA) or 40 pmol l^{-1} ^{3}H-labelled BQ-123 (cyclo[-D-Asp–L-Pro–D-Val–L-Leu–D-Trp-]), a selective endothelin ET_A receptor antagonist (Amersham, Buckinghamshire, England) were added and incubated in a volume of 0.5 ml. Dissociation time courses were studied by adding endothelin-1, PD 155080 and A-192621 after the binding had reached equi-

Table 1
Effects of endotoxin infusion started after baseline measurements at time zero (0 h). After 2 h of endotoxemia, one group ($n = 3–6$) received A-192621 in combination with PD 155080 and another group ($n = 5–6$) only A-192621. Control animals ($n = 4–6$) only received endotoxin and vehicle. Data are compared between groups before and after administration of either A-192621 and PD 155080 in combination or only A-192621. Data are presented as mean ± S.E.M.

Parameter	Group	0 h	2 h	5 h
Arterial oxygen tension (kPa)	Controls	21.7 ± 0.9	16.0 ± 1.7[a]	13.6 ± 1.7
	A-192621/PD 155080	19.9 ± 1.21	16.3 ± 0.62[a]	15.7 ± 0.95
	A-192621	18.7 ± 1.35	15.4 ± 86[a]	
Arterial oxygen saturation (%)	Controls	99 ± 0	98 ± 1[a]	91 ± 6
	A-192621/PD 155080	99 ± 0	98 ± 0[a]	98 ± 0
	A-192621	99 ± 0	97 ± 0[a]	
Arterial carbon dioxide tension (kPa)	Controls	4.7 ± 0.37	5.2 ± 0.23[b]	6.4 ± 0.5[c]
	A-192621/PD 155080	4.5 ± 0.32	5.5 ± 0.23[b]	5.3 ± 0.14[c]
	A-192621	5.1 ± 0.28	5.8 ± 0.21[b]	
Pulmonary shunt fraction (%)	Controls	3 ± 1	12 ± 4[a]	15 ± 6
	A-192621/PD 155080	4 ± 1	9 ± 2[a]	12 ± 2
	A-192621	5 ± 1	10 ± 2[a]	
Central venous pressure (mm · Hg^{-1})	Controls	4 ± 1.8	4 ± 1.8	3 ± 1.3
	A-192621/PD 155080	2 ± 1.0	2 ± 1.0	2 ± 1.1
	A-192621	2 ± 0.7	2 ± 1.1	
Pulmonary capillary wedge pressure (mm · Hg^{-1})	Controls	3 ± 1.4	6 ± 1.7[a]	8 ± 1.2
	A-192621/PD 155080	3 ± 1.1	5 ± 1.0[a]	3 ± 0.9[d]
	A-192621	4 ± 0.7	6 ± 1.2[a]	
Left ventricular stroke work index (g · m · kg^{-1})	Controls	1.50 ± 0.18	0.47 ± 0.08[a]	0.28 ± 0.10
	A-192621/PD 155080	1.32 ± 0.17	0.32 ± 0.06[a]	0.39 ± 0.08[e]
	A-192621	1.47 ± 0.23	0.26 ± 0.05[a]	
Right ventricular stroke work index (g · m · kg^{-1})	Controls	0.24 ± 0.04	0.20 ± 0.03[a]	0.15 ± 0.02
	A-192621/PD 155080	0.25 ± 0.02	0.24 ± 0.05[a]	0.17 ± 0.03
	A-192621	0.29 ± 0.04	0.20 ± 0.03[a]	
Arterial lactate (mmol · l^{-1})	Controls	1.1 ± 0.3	2.7 ± 0.5[a]	3.5 ± 1.4
	A-192621/PD 155080	1.4 ± 0.1	3.0 ± 0.6[a]	2.2 ± 0.9
	A-192621	1.4 ± 0.5	3.0 ± 0.6[a]	
Haemoglobin concentration (g · l^{-1})	Controls	111 ± 4	129 ± 5[a]	126 ± 3
	A-192621/PD 155080	119 ± 5	114 ± 7[a]	110 ± 7
	A-192621	112 ± 3	107 ± 6[a]	
Erythrocyte volume fraction (%)	Controls	34 ± 2	38 ± 2[a]	39 ± 2
	A-192621/PD 155080	37 ± 2	35 ± 2[a]	34 ± 3
	A-192621	34 ± 1	32 ± 2[a]	

[a] Significant changes over time, prior to intervention $P < 0.001$.
[b] Significant changes over time, prior to intervention $P < 0.01$.
[c] Significant changes over time, after intervention $P < 0.05$.
[d] Significant changes between groups after intervention $P < 0.001$.
[e] Significant changes between groups after intervention $P < 0.01$.

librium. Non-specific binding was determined by adding unlabelled endothelin-1 (0.1 μmol l^{-1}) to the membranes before the addition of radioligand. Bound and free ligands were separated by rapid centrifugation. Pellets were washed once with assay buffer, dissolved in water and transferred to new test tubes. Radioactivity in both free and bound fractions was counted in a LKB 1272 CliniGamma Counter (Wallac, Finland) or Beta counter (Beckman-3801, Bromma, Sweden).

2.7. In vivo characterisation

Five animals were anaesthetised as described above. After a midline incision, an ultrasonic flow probe was placed around the splenic artery in two pigs and around the portal vein in three pigs. The flow probes were connected to a flowmeter (T-202-s, Transonic Systems , NY, USA). After baseline measurements, the animals received either an endothelin-1 infusion (American Peptide, CA, USA) of 120 pmol × kg^{-1} (for PD 155080 characterisation) or the selective endothelin ET_B receptor agonist sarafotoxin 6c (American Peptide, CA, USA) of 140 × pmol kg^{-1} (for A-192621 characterisation). At the end of the endothelin-1 infusion, an arterial blood sample for analysis of endothelin-1-like immunoreactivity was obtained. Following a resting period that allows blood flows to return to baseline values, the animals received a bolus infusion of either PD 155080 (10 mg kg^{-1}) or A-192621 (10 mg kg^{-1}) after which endothelin-1 or sarafotoxin 6c infusions were repeated in the same fashion as before antagonist administration.

2.8. Statistics

Data are presented as mean (S.E.M.). An univariate analysis for repeated measures of variance (ANOVA) (Statistica 5.0, StatSoft, Tulsa, USA) was used for statistical calculation of changes over time and for comparison between the control group and the intervened groups, before intervention (T_0–T_2) and after intervention ($T_{2,5}$ (or T_3)–T_5). T_2 was used as covariate for the post intervention ANOVA. Differences were considered significant at $P < 0.05$.

3. Results

Prior to intervention, no differences were found in any of the measured or calculated parameters between the groups.

The animals only receiving A-192621 all died within 1 (four animals) to 2 (two animals) h after administration of the drug. Therefore, statistical comparisons after intervention were only made between the control group and the group receiving the combination of A-192621 and PD 155080.

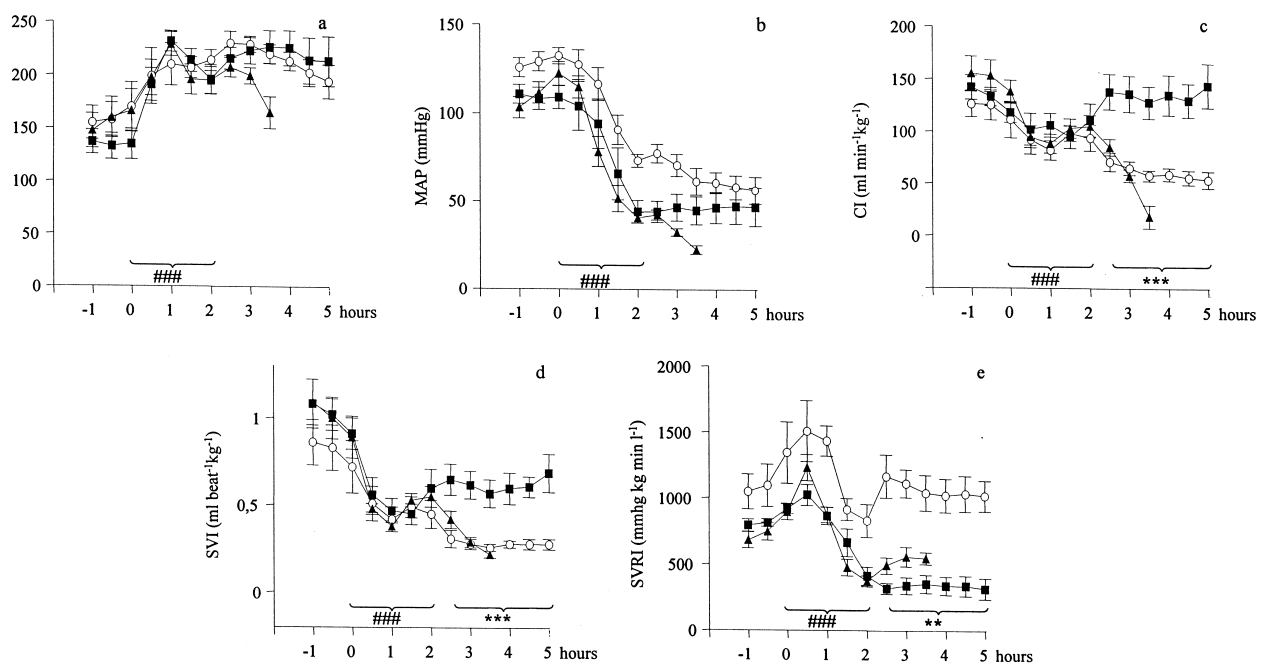

Fig. 2. Heart rate (HR) (a) mean arterial blood pressure (MAP) (b) cardiac index (CI) (c) stroke volume index (SVI) (d) and systemic vascular resistance (SVRI) (e) during endotoxin infusion, started at 0h. At 2h one group ($n = 6$) (▲) received an intravenous bolus of A-192621 (10 mg kg^{-1}) followed by a continuous infusion (5 mg $kg^{-1}h^{-1}$). Another group ($n = 6$) (■) received A-192621 in the same fashion in combination with a bolus of PD 155080 (10 mg kg^{-1}) followed by a continuous infusion (5 mg $kg^{-1}h^{-1}$). Control pigs ($n = 6$) (○) only received endotoxin infusion and vehicle. $^{###}P < 0.001$ for differences in time before intervention, $^{**}P < 0.01$ and $^{***}P < 0.001$ for differences between groups after intervention.

3.1. Pulmonary circulation and gas exchange

A biphasic increase in mean pulmonary artery pressure and pulmonary vascular resistance index was seen in response to endotoxin. The second, more prolonged increase in these parameters in the control animals was completely and rapidly counteracted by A-192621/PD 155080 (Figs. 1a and b) whereas A-192621 alone appeared to be without effect on the pulmonary hypertension.

PaO_2 and SaO_2 decreased and $PaCO_2$ increased in response to endotoxin (2 h) (Table 1). This decrease in PaO_2 was sustained throughout the experiment while $PaCO_2$ was further increased. Neither parameter was affected by A-192621/PD 155080 (Table 1). The pulmonary shunt fraction increased after onset of endotoxin infusion

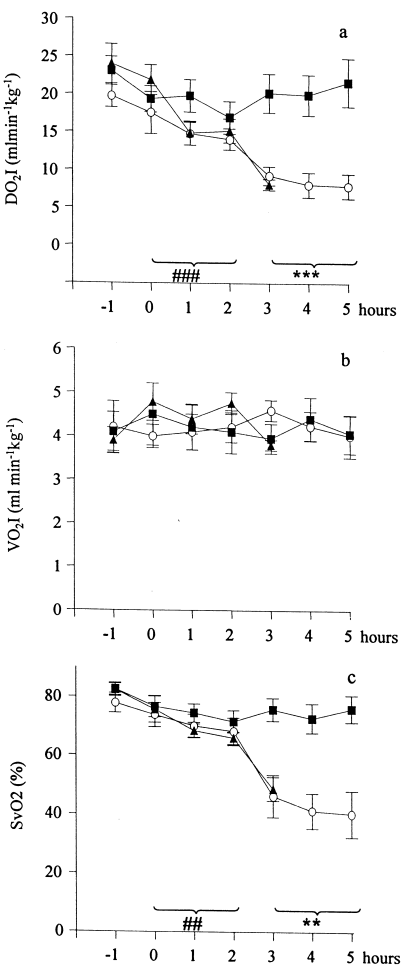

Fig. 3. Systemic oxygen delivery index (DO_2I) (a) systemic oxygen consumption index (VO_2I) (b) and mixed venous oxygen saturation (SvO_2) (c) during endotoxin infusion, started at 0 h. At 2 h one group ($n=6$) (▲) received an intravenous bolus of A-192621 (10 mg kg^{-1}) followed by a continuous infusion (5 mg kg^{-1} h^{-1}). Another group ($n=6$) (■) received A-192621 in the same fashion in combination with a bolus of PD 155080 (10 mg kg^{-1}) followed by a continuous infusion (5 mg kg^{-1} h^{-1}). Control pigs ($n=6$) (○) only received endotoxin infusion and vehicle. $^{\#\#}P<0.01$ and $^{\#\#\#}P<0.001$ for differences in time before intervention, $^{**}P<0.01$ $^{***}P<0.001$ for differences between groups after intervention.

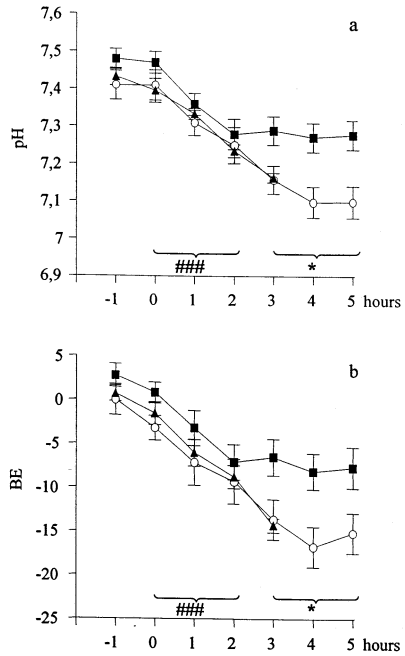

Fig. 4. pH (a) and base excess (BE) (b) during endotoxin infusion, started at 0h. At 2h one group ($n=6$) (▲) received an intravenous bolus of A-192621 (10 mg kg^{-1}) followed by a continuous infusion (5 mg kg^{-1} h^{-1}). Another group ($n=6$) (■) received A-192621 in the same fashion in combination with a bolus of PD 155080 (10 mg kg^{-1}) followed by a continuous infusion (5 mg kg^{-1} h^{-1}). Control pigs ($n=6$) (○) only received endotoxin infusion and vehicle. $^{\#\#\#}P<0.001$ for differences in time before intervention, $^{*}P<0.05$ for differences between groups after intervention.

but after T_2, no further increase was seen in controls or in pigs receiving A-192621/PD 155080 (Table 1).

3.2. Systemic haemodynamics

After onset of endotoxin infusion, heart rate increased while mean arterial blood pressure, cardiac index, and stroke volume index decreased (Fig. 2a, b, c and d). Intervention with A-192621/PD 155080 at T_2 counteracted the decrease in cardiac index and stroke volume index (Fig. 2c and d). Heart rate and mean arterial blood pressure were not significantly changed in either group the last 3 h of the experiment (Fig. 2a and b). After administration, heart rate, mean arterial blood pressure, cardiac index and stroke volume index all fell to very low values among the animals only receiving A-192621 (Fig. 2a, b, c and d). Central venous pressure was not changed in any group during the experiment while pulmonary capillary wedge pressure initially increased. This increase in pulmonary capillary wedge pressure was counteracted in the group receiving A-192621/PD 155080 (Table 1). There was an initial transient increased in systemic vascular resistance index in all groups in response to endotoxin. In the control group systemic vascular resistance index returned to baseline values while A-192621/PD 155080 treatment resulted in a further decrease with a significant

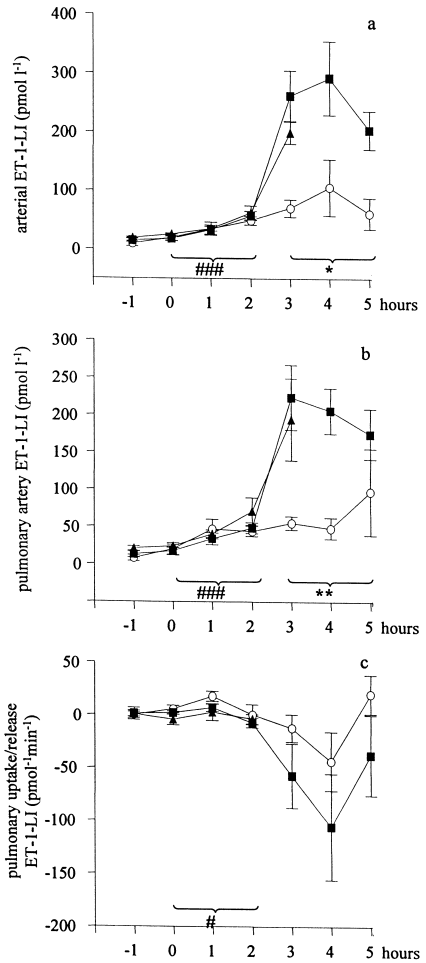

Fig. 5. Arterial plasma endothelin-1-like immunoreactivity levels (a) pulmonary artery plasma endothelin-1-like immunoreactivity levels (b) and pulmonary uptake or release of endothelin-1-like immunoreactivity (c) where a negative value indicates net production and a positive value net uptake, during endotoxin infusion started at 0 h. At 2 h one group ($n = 3$–5) (▲) received an intravenous bolus of A-192621 (10 mg kg^{-1}) followed by a continuous infusion (5 mg kg^{-1} h^{-1}). Another group ($n = 5$–6) (■) received A-192621 in the same fashion in combination with a bolus of PD 155080 (10 mg kg^{-1}) followed by a continuous infusion (5 mg kg^{-1} h^{-1}). Control pigs ($n = 4$–5) (○) only received endotoxin infusion and vehicle. $^{\#}P < 0.05$ and $^{\#\#\#}P < 0.001$ for differences in time before intervention, $^{*}P < 0.05$ and $^{**}P < 0.01$ for differences between groups after intervention.

difference between groups (Fig. 2e). Both left ventricular stroke work index and right ventricular stroke work index markedly decreased during the first 2 h of endotoxemia. Left ventricular stroke work index was slightly higher in the A-192621/PD 155080 group after intervention while no significant inter-group differences in right ventricular stroke work index was observed (Table 1). Systemic oxygen delivery index progressively decreased during endotoxaemia in all groups while systemic oxygen consumption index was unchanged. Intervention with A-192621/PD 155080 significantly increased systemic oxygen delivery index compared to controls but did not affect systemic oxygen consumption index (Fig. 3a and b). SvO_2 significantly decreased the first 2 h while administration of A-192621/PD 155080 resulted in higher values compared to untreated animals (Fig. 3c).

3.3. Acid–base status and haemoglobin concentration

Both arterial pH and base excess decreased markedly during endotoxaemia. A-192621/PD 155080 prevented any further decrease resulting in higher pH and base excess compared to the control group (Fig. 4a and b). The A-192621 treated animals had pH and base excess values in the same range as the control animals. Arterial lactate levels increased in all groups during the first 2 h. Despite a tendency towards lower lactate levels, A-192621/PD 155080 administration did not result in significant differences between the groups (Table 1). Endotoxaemia resulted in an initial haemoconcentration with increased haemoglobin levels and erythrocyte volume fraction followed by a gradual return to pre-endotoxin values in both groups with a tendency towards higher values among control animals (Table 1).

3.4. Endothelin-1

The initial 2 h of endotoxaemia caused a three-fold increase in arterial plasma endothelin-1-like immunoreactivity levels in all groups. Intervention with A-192621/PD 155080 resulted in a further marked increase in arterial plasma endothelin-1-like immunoreactivity levels. The values at T_5 were four times higher in this group compared to controls (Fig. 5a). Endothelin-1-like immunoreactivity lev-

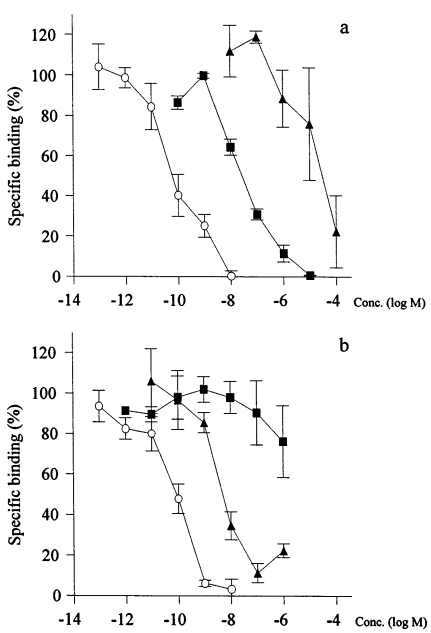

Fig. 6. Specific binding in percent of ^{3}H-labelled BQ 123 (a) and ^{125}I-labelled IRL 1620 (b) in membrane preparations from porcine heart in response to incremental doses of endothelin-1(○), A-192621(▲) and PD 155080(■).

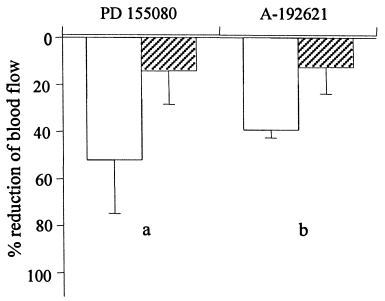

Fig. 7. Reduction in percent of baseline values of splenic artery blood flow ($n = 2$) (a) in and of portal blood flow ($n = 3$) (b). Response to an endothelin-1 infusion of 120 pmol·kg^{-1} before (open bars) and after (hatched bars) administration of PD 155080 (10 mg kg^{-1})(a) and to a sarafotoxin 6c infusion of 140 pmol·kg^{-1} before (open bars) and after (hatched bars) administration of A-192621 (10 mg kg^{-1})(b).

els also increase to similar values up until T_3 among the A-192621 treated group (Fig. 5a). A similar pattern was seen in pulmonary arterial endothelin-1-like immunoreactivity plasma levels with higher endothelin-1-like immunoreactivity levels in the treated group (Fig. 5b). No significant pulmonary net uptake or release of endothelin-1-like immunoreactivity was seen, although a tendency for release was observed after A-192621/PD 155080 administration (Fig. 5c).

3.5. Receptor characterisations

Both A-192621 and PD 155080 showed a high degree of selectivity for the endothelin ET_B and endothelin ET_A receptor respectively. IC_{50} values for A-192621 was 3.0×10^{-5} M for the endothelin ET_A receptor and 5.0×10^{-9} M for the endothelin ET_B receptor. For PD 155080, the IC_{50} value was 2.6×10^{-8} M for the endothelin ET_A receptor and was not reached at concentrations up to 1.0×10^{-6} for the endothelin ET_B receptor. In comparison, endothelin-1 had IC_{50} values of 6.0×10^{-11} for the endothelin ET_A receptor and 8.5×10^{-11} for the endothelin ET_B receptor (Fig 6a and b).

3.6. In vivo characterisation

Endothelin-1 infusion resulted in a decrease in splenic blood flow by 52%. After treatment with PD 155080, endothelin-1 infusion reduced splenic blood flow by only 14% (Fig. 7a). Sarafotoxin 6c caused a decrease in portal blood flow by 39% which, after administration of A-192621, was limited to 13% (Fig. 7b).

4. Discussion

The major findings in the present study are the pronounced cardiovascular and metabolic effects of endothelin receptor antagonism given during porcine endotoxin shock and the marked differences in response to selective endothelin ET_B versus mixed endothelin ET_B/ET_A receptor antagonism. Selective endothelin endothelin$_B$ receptor antagonism alone has deleterious effects while the combination of selective endothelin ET_A and endothelin ET_B receptor antagonism shows strong favourable effects on cardiopulmonary circulation and metabolic condition.

Endothelin ET_B receptor antagonism resulted in a cardiovascular collapse and all animals died within 2 h after administration. Several possible mechanisms behind the fatal effects may be discussed. Endothelin ET_B receptor stimulation may mediate both vasodilation and vasoconstriction and this receptor subtype is also suggested to be responsible for clearance of circulating endothelin-1 (Dupuis et al., 1996). Therefore, antagonism of this receptor generates an increase in plasma endothelin-1 levels, which may lead to increased endothelin ET_A receptor activation and pronounced vasoconstriction. The decrease in cardiac output, caused by endothelin-1 that is seen under various circumstances in animals as well as in man, has been suggested to be due to endothelin ET_A receptor-mediated coronary vasoconstriction (Kiely et al., 1997; Szalay et al., 1997) whereas the endothelin ET_B receptor has been proposed to cause an increase in inotropy (Beyer et al., 1995). Selective endothelin ET_B receptor antagonism may therefore have negative effects on cardiac contractility and also, the coronary constrictive effect of the endothelin ET_A receptor might be further potentiated analogously to the discussion above. Some studies support the existence of cross talk between the endothelin ET_A and endothelin ET_B receptors (Mickley et al., 1997; Ozaki et al., 1997). Each receptor could compensate for each other, i.e., selective endothelin ET_B receptor antagonism would lead to a more pronounced effect of endothelin ET_A receptor activation, contributing to the negative effect of selective endothelin ET_B receptor antagonism seen in this study. Another issue is the route of administration of the antagonist. Since the vasodilating endothelin ET_B receptors are located on the endothelium, these receptors will most likely to be reached first when an i.v. route is chosen. Antagonism of these receptors, mediating vasodilation through release of NO and prostacyclin could therefore contribute to the increase in afterload and cardiovascular collapse. A direct toxic effect of A-192621 is unlikely since the combination of A-192621 and PD 155080 was beneficial.

Endotoxin induced-pulmonary hypertension is a reproducible finding in various animal models (Leeper-Woodford et al., 1991; Weitzberg et al., 1993) and is also a feature of human sepsis where it may affect right ventricular function (Vincent, 1998). Mechanisms such as vasoconstriction and leukocyte aggregation have been suggested (Bigatello and Zapol, 1996) and endothelin-1 has been shown to cause such changes (Helset et al., 1994; Curzen et al., 1996). The second increase in mean pulmonary artery pressure and pulmonary vascular resistance

index was completely counteracted by combined endothelin ET_A/ET_B receptor antagonism. We have previously shown that selective endothelin ET_A receptor antagonism alone has the ability to reverse endotoxin-induced pulmonary hypertension in supporting that endothelin ET_A receptor activation contributes to this increase in pulmonary vascular tone (Wanecek et al., 1999). Despite this we could not exclude some contribution to the pulmonary hypertension by endothelin ET_B receptor activation which has been suggested by some authors (Fukuroda et al., 1996). However, this mechanism seems to be of less importance in this model, since A-192621 appeared to be without effect on these parameters. Pulmonary gas exchange decreased after onset of endotoxin infusion reflected by an increase in $PaCO_2$ and decreased SaO_2 and PaO_2 as well as an increase in pulmonary shunt fraction. Despite tendencies towards an improvement, by endothelin ET_A/ET_B receptor antagonism, no significant changes in these parameters was obtained. Hypoxia-induced pulmonary vasoconstriction in a similar porcine model has been suggested to be endothelin ET_A receptor mediated (Holm et al., 1998). In this study as well in studies using selective endothelin ET_A receptor antagonism (Wanecek et al., 1999), no effect on PaO_2 was obtained. Therefore hypoxia-induced pulmonary vasoconstriction appears to be of less importance in the present endotoxin model. The decrease in SvO_2, mainly reflecting cardiac index, seen among control animals was prevented by A-192621/PD 155080 administration. Absence of an increased pulmonary shunt fraction from combined endothelin ET_A/ET_B receptor antagonism indicates an unaffected ventilation/perfusion ratio upon A-192621/PD 155080 administration.

The effect of combined A-192621 and PD 155080 treatment on the cardiovascular system during endotoxin infusion is quite different compared to either drug used alone (Wanecek et al., 1999). After administration of both antagonists in combination, cardiac index increased to baseline values within 30 min, and was due to the increased stroke volume index since heart rate was unchanged. The issue of sepsis/endotoxin induced cardiac depression is still under debate. Advocated mechanisms include the release of myocardial depressant factors such as tumour necrosis factor-α (TNF-α), interleukin-1, NO (Herbertson et al., 1996; Kumar et al., 1996) and, although less forwarded in human septic shock, coronary artery vasoconstriction (Peyton et al., 1976). TNF-α as well as endothelin-1 causes coronary artery vasoconstriction during endotoxic conditions and the vasoconstrictive properties of TNF-α has been suggested to be mediated by endothelin-1 (Hohlfeld et al., 1995). Although indications of the existence of coronary constrictive endothelin ET_B receptors has been shown in dogs with heart failure (Cannan et al., 1996) the endothelin ET_A receptor has been suggested to be the major coronary vasoconstrictive endothelin receptor (Wang et al., 1995). We have previously shown that selective endothelin ET_A receptor antagonism by PD 155080 is without beneficial effects on cardiac performance during porcine endotoxin shock (Wanecek et al., 1999). The markedly improved cardiac index and stroke volume index from combined endothelin ET_A/ET_B receptor antagonism might be explained by a larger reduction in both right- and left-sided afterload compared to selective endothelin ET_A receptor antagonism. This is probably due to the large vascular population of constrictive endothelin ET_B receptors (Lodge et al., 1995). Afterload reduction has also been forwarded as an explanation behind the increased cardiac output seen from mixed endothelin antagonism during human congestive heart failure (Kiowski et al., 1995). Also, the left ventricular stroke work index actually increased among the A-192621/PD 155080 treated animals. Meanwhile, the right ventricular stroke work index did not differ between groups which to a certain degree speaks against a negative inotropic effect of combined endothelin ET_A/ET_B receptor antagonism, which has been described by others (Ishikawa et al., 1988; Mebazaa et al., 1993). Therefore, this study together with our previous study using PD 155080 alone (Wanecek et al., 1999), strongly suggests that dual endothelin ET_A/ET_B receptor antagonism is necessary to obtain favourable effects. Mean arterial blood pressure did not significantly decrease upon mixed endothelin ET_A/ET_B antagonism despite a marked decrease in systemic vascular resistance, a result of the improvement in cardiac index. As a consequence of the increased cardiac index, systemic oxygen delivery index significantly increased after administration of A-192621/PD 155080. This did not result in an overall increase in oxygen consumption. However, lack of changes in global systemic oxygen consumption index does not rule out a more favourable local oxygen utilisation. Antagonism of the vasoconstrictive properties of vessels in vital organs such as the heart, brain, splanchnicus and kidneys could direct blood flow from skeletal muscle and skin. Also, oxygen is used for purposes other then oxidative phosphorylation like the formation of reactive oxygen species seen during endotoxaemia, consuming large amounts of oxygen (Vlessis et al., 1995). Interestingly, endothelin-1 has been shown to promote leukocyte activation (Caramelo et al., 1997) resulting in production of reactive oxygen species and therefore endothelin receptor antagonism might result in better oxygen utilisation. In fact, the lesser degree of metabolic acidosis, reflected in a higher pH and base excess and a tendency towards lower arterial plasma lactate levels, among A-192621/PD 155080 treated animals suggests a more adequate oxygen utilisation in response to combined endothelin ET_A/ET_B receptor antagonism.

Both arterial and pulmonary artery plasma endothelin-1-like immunoreactivity levels increased in response to endotoxaemia. These results are in line with earlier studies in both experimental and human sepsis (Weitzberg et al., 1991; Curzen et al., 1997). After administration of com-

bined endothelin ET_A/ET_B receptor antagonism, a further elevation in plasma endothelin-1-like immunoreactivity levels were seen analogous with previous results from our group (Wanecek et al., 1997b) and others (Dupuis et al., 1996) and is thought to be caused by antagonism of the clearing function of endothelin ET_B receptors. This is also supported in this study where selective endothelin ET_B receptor antagonism by A-192621 increased plasma endothelin-1-like immunoreactivity levels to values similar to those in the A-192621/PD 155080 group. The pulmonary circulation has been suggested to be an important producer of endothelin-1 during diseased conditions (Stewart et al., 1991). In the present study, a tendency towards net pulmonary endothelin-1 release was seen, which to some degree supports the concept of the pulmonary circulation as a net producer of endothelin-1 during endotoxaemia. Another supportive finding for a net release is the fact that during endothelin-1 infusion in the pig, a pulmonary uptake is seen (Weitzberg et al., 1995). If other sources of endothelin-1 production are of importance during endotoxaemia remains to be elucidated.

In vitro receptor characterisation on membrane preparations from porcine heart shows that both antagonists used in this study have high selectivity for its receptor. In addition, the in vivo effect of each drug was studied. The splenic artery and portal vein are known to respond strongly to endothelin-1 and sarafotoxin 6c, respectively, suggesting high population of endothelin ET_A/ET_B receptors (Hemsen, 1991; Filippelli et al., 1996). Arterial plasma endothelin-1-like immunoreactivity levels after endothelin-1 infusion were similar to those seen during endotoxaemia among the control animals (not shown). Both PD 155080 and A-192621 counteracted the vasoconstrictive properties of endothelin-1 and sarafotoxin 6c, respectively, indicating that both drugs affects the receptor activation in these vascular beds. Together this suggests that both PD 155080 and A-192621 are suitable substances for in vivo studies of both endothelin ET_A/ET_B receptor mediated-mechanisms.

In conclusion, the present study shows that the endothelin system is of importance in the cardiopulmonary vascular pathophysiology of porcine endotoxin shock. In this model, combination of a selective endothelin ET_A and a selective endothelin ET_B receptor antagonist markedly improved pulmonary circulation, cardiac performance and the metabolic condition. In bright contrast, selective endothelin ET_B receptor antagonist alone had detrimental effects resulting in cardiovascular collapse. A possible mechanism is increased endothelin ET_A receptor-mediated events due to decreased clearance from endothelin ET_B receptors. Previous results in an identical model using selective endothelin ET_A receptor antagonism showed effects mainly limited to the pulmonary circulation. These results show the importance of the balance between the different endothelin receptor subtypes and that this has to be taken in consideration when discussing interference of the endothelin system as a treatment modality.

Acknowledgements

We are very grateful to Jerry Wessale (Abbott Laboratories) and Mrs. Anette Doherty (Parke–Davis) for the generous gifts of A-192621 and PD 155080, respectively. For valuable technical assistance we are grateful to Mrs. Carina Nihlén and Miss Margareta Stensdotter.

This study was financially supported by Swedish Medical Research Council (no. 04X-12586-01A 10354,), Laeardal Foundation, Swedish Society for Medical Research, Magn Bergvall Foundation, Åke Wibergs Foundation, Swedish Heart–Lung Found and funds from Karolinska Institute.

References

Bassenge, E., 1996. Endothelial function in different organs. Prog. Cardiovasc. Dis. 39, 209–228.

Battistini, B., Forget, A.-M., Laight, D., 1996. Potential roles for endothelins in systemic inflammatory response syndrome with a particular relation to cytokines. Shock 5, 167–183.

Beyer, M.E., Slesak, G., Hoffmeister, H.M., 1995. In vivo hemodynamic and inotropic effects of the endothelinB agonist IRL 1620. J. Cardiovasc. Pharmacol 26 (Suppl. 3), S190–S192.

Bigatello, L., Zapol, M., 1996. New approaches to acute lung injury. Britt. J. Anaesth. 77, 99–109.

Cannan, C.R., Burnett, J.C. Jr., Lerman, A., 1996. Enhanced coronary vasoconstriction to endothelin-B-receptor activation in experimental congestive heart failure. Circulation 93, 646–651.

Caramelo, C., Lopez Farre, A., Riesco, A., Casado, S., 1997. Role of endothelin-1 in the activation of polymorphonuclear leukocytes. Kidney Int. 52, 56–59.

Curzen, N.P., Mitchell, J.A., Jourdan, K.B., Griffiths, M.J, Evans, T.W., 1996. Endothelin-1-induced contraction of pulmonary arteries from endotoxemic rats is attenuated by the endothelin-A receptor antagonist, BQ123. Crit. Care Clin. 24, 2007–2013.

Curzen, N.P., Kaddoura, S., Griffiths, M.J., Evans, T.W., 1997. Endothelin-1 in rat endotoxemia: mRNA expression and vasoreactivity in pulmonary and systemic circulations. Am. J. Physiol. 272, H2353–H2360.

De Nucci, G., Thomas, R., d'0rleans-Juste, P., 1988. Pressor effects of circulating endothelin are limited by its removal in the pulmonary circulation and by the release of prostacyklin and endothelium-derived relaxing factor. Proc. Natl. Acad. Sci. U.S.A. 85, 9797–9800.

Druml, W., Steltzer, H., Waldhausl, W., Lenz, K., Hammerle, H., Vierhapper, A., Gasic, S., Wagner, O., 1993. Endothelin-1 in adult respiratory distress syndrome. Am. Rev. Respir. Dis. 65 (148), 1169–1173.

Dupuis, J., Goresky, C.A., Fournier, A., 1996. Pulmonary clearance of circulating endothelin-1 in dogs in vivo: exclusive role of ETB receptors. J. Appl. Physiol. 81, 1510–1515.

Ehrenreich, H., Schilling, L., 1995. New developments in the understanding of cerebral vasoregulation and vasospasm: the endothelin–nitric oxide network. Cleveland Clin. Q. 62, 105–116.

Elshourbagy, N.A., Adamou, J.E., Gagnon, A.W., Wu, H.L., Pullen, M., Nambi, P., 1996. Molecular characterization of a novel human endothelin receptor splice variant. J. Biol. Chem. 271, 25300–25307.

Filippelli, A., Falciani, M., Palla, A., D'Amico, M., Vacca, C., Rossi, F., 1996. Distribution of endothelin-1-receptor subtypes in rat portal vein. J. Cardiovasc. Pharmacol. 27, 113–118.

Fukuroda, T., Ozaki, S., Ihara, M., Ishikawa, K., Yano, M., Miyauchi, T., Ishikawa, S., Onizuka, M., Goto, K., Nishikibe, M., 1996. Necessity

of dual blockade of endothelin ET_A and ET_B receptor subtypes for antagonism of endothelin-1-induced contraction in human bronchi. Br. J. Pharmacol. 117, 995–999.

Goldie, R.G., Knott, P.G., Carr, M.J., Hay, D.W., Henry, P.J., 1996. The endothelins in the pulmonary system. Pulm. Pharmacol. 9, 69–93.

Helset, E., Ytrehus, K., Tveita, T., Kjëve, J., Jörgensen, L., 1994. ET-1 causes accumulation of leukocytes in the pulmonary circulation. Circ. Shock 44, 201–209.

Hemsen, A., 1991. Biochemical and functional characterization of endothelin peptides with special references to vascular effects. Acta. Physiol. Scand. 142 (Suppl. 602), 5–61.

Herbertson, M.J., Werner, H.A., Walley, K.R., 1996. Nitric oxide synthase inhibition partially prevents decreased LV contractility during endotoxemia. Am. J. Physiol. 270, H1979–H1984.

Hohlfeld, T., Klemm, P., Thiemermann, C., Warner, T.D., Schror, K., 1995. The contribution of tumour necrosis factor — alpha and endothelin-1 to the increase of coronary resistance in hearts from rats treated with endotoxin. Br. J. Pharmacol. 116, 3309–3315.

Holm, P., Liska, J., Franco-Cereceda, A., 1998. The ET_A receptor antagonist, BMS-182874, reduces acute hypoxic pulmonary hypertension in pigs in vivo. Cardiovasc. Res. 37, 765–771.

Ishikawa, T., Yanagisawa, M., Kimura, S., Goto, K., Masaki, T., 1988. Positive inotropic actions of novel vasoconstrictor peptide endothelin on guinea pig atria. Am. J. Physiol. 255, H970–H973.

Kiely, D.G., Cargill, R.I., Struthers, A.D., Lipworth, B.J., 1997. Cardiopulmonary effects of endothelin-1 in man. Cardiovasc. Res. 33, 378–386.

Kiowski, W., Sütsch, G., Hunziker, P., Müller, P., Kim, J., Oechslin, E., Schmitt, R., Jones, R., Bertel, O., 1995. Evidence of ET-1-mediated vasoconstriction in severe chronic heart failure. Lancet 346, 732–736.

Kumar, A., Thota, V., Dee, L., Olson, J., Uretz, E., Parrillo, J.E., 1996. Tumor necrosis factor alpha and interleukin 1beta are responsible for in vitro myocardial cell depression induced by human septic shock serum. J. Exp. Med. 183, 949–958.

Leeper-Woodford, S., Carey, P., Byrne, K., Fisher, J., Blocher, C., Sugerman, H., Fowler, A. III, 1991. Ibuprofen attenuates plasma tumor necrosis factor activity during sepsis-induced acute lung injury. J. Appl. Physiol. 71, 915–923.

Lodge, N.J., Zhang, R., Halaka, N.N., Moreland, S., 1995. Functional role of endothelin ET_A and ET_B receptors in venous and arterial smooth muscle. Eur. J. Pharmacol. 287, 279–285.

MacLean, M.R., Mackenzie, J.F., Docherty, C.C., 1998. Heterogeneity of endothelin-B receptors in rabbit pulmonary resistance arteries. J. Cardiovasc. Pharmacol. 31 (Suppl. 1), S115–S118.

Mebazaa, A., Mayoux, E., Maeda, K., Martin, L.D., Lakatta, E.G., Robotham, J.L., Shah, A.M., 1993. Paracrine effects of endocardial endothelial cells on myocyte contraction mediated via endothelin. Am. J. Physiol. 265, H1841–H1846.

Miasiro, N., Karaki, H., Paiva, A.C., 1998. Distinct endothelin-B receptors mediate the effects of Sarafotoxin S6c and IRL1620 in the ileum. J. Cardiovasc. Pharmacol. 31 (Suppl. 1), 175–178.

Mickley, E.J., Gray, G.A., Webb, D.J., 1997. Activation of endothelin ET_A receptors masks the constrictor role of endothelin ET_B receptors in rat isolated small mesenteric arteries. Br. J. Pharmacol. 120, 1376–1382.

Ozaki, S., Ohwaki, K., Ihara, M., Ishikawa, K., Yano, M., 1997. Coexpression studies with endothelin receptor subtypes indicate the existence of intracellular cross-talk between ET_A and ET_B receptors. J. Biochem. (Tokyo) 121, 440–447.

Parillo, J., Parker, M., Natanson, C., Suffredini, A., Danner, R., Cunnion, R., Ognibene, F., 1990. Septic shock in humans. Ann. Intern. Med. 113, 227–242.

Pernow, J., Wang, Q.D., 1997. Endothelin in myocardial ischaemia and reperfusion. Cardiovasc. Res. 33, 518–526.

Peyton, M.D., Hinshaw, L.B., Greenfield, L.J., Elkins, R.C., 1976. The effects of coronary vasodilatation on cardiac performance during endotoxin shock. Surg. Gynecol. Obstet. 143, 533–538.

Piero, C., 1995. Influence of endothelium on cultured vascular smooth muscle cell proliferation. Hypertension 25, 748–751.

Pittet, J., Morel, D., Hemsen, A., Gunning, K., Lacroix, J.-S., Suter, P., Lundberg, J.M., 1991. Elevated plasma endothelin-1 concentrations are associated with the severity of illness in patients with sepsis. Ann. Surg. 213, 261–264.

Reeder, L.B., Ferguson, M.K., 1996. Endothelin-1 synthesis and receptor-mediated activity in porcine lymph vessels. J. Surg. Res. 63, 215–219.

Sakurai-Yamashita, Y., Yamashita, K., Yoshida, A., Obana, M., Takada, K., Shibaguchi, H., Shigematsu, K., 1997. Rat peritoneal macrophages express endothelin ET_B but not endothelin ET_A receptors. Eur. J. Pharmacol. 338, 199–203.

Shindo, T., Kurihara, H., Kurihara, Y., Morita, H., Yazaki, Y., 1998. Upregulation of endothelin-1 and adrenomedullin gene expression in the mouse endotoxin shock model. J. Cardiovasc. Pharmacol. 31 (Suppl. 1), S541–S544.

Stewart, D., Levy, R., Cernacek, P., Langleben, D., 1991. Increased plasma endothelin-1 in pulmonary hypertension: marker of mediator of disease? Ann. Intern Med. 114, 464–469.

Szalay, L., Boros, M., Baranyi, L., Okada, H., Nagy, S., 1997. Endothelin-1-induced circulatory response in the rat: the role of ET_A and ET_B receptors. Acta. Chim. Hung. 36, 340–342.

Wanecek, M., Oldner, A., Rudehill, A., Sollevi, A., Alving, K., Weitzberg, E., 1997a. Cardiopulmonary dysfunction during porcine endotoxin shock is effectively counteracted by the endothelin receptor antagonist bosentan. Shock 7, 364–370.

Wanecek, M., Rudehill, A., Hemsen, A., Lundberg, J.M., Weitzberg, E., 1997b. The endothelin receptor antagonist, bosentan, in combination with the cyclooxygenase inhibitor, diclofenac, counteracts pulmonary hypertension in porcine endotoxin shock. Crit. Care. Clin. 25, 848–857.

Wanecek, M., Oldner, A., Rudehill, A., Sollevi, A., Alving, K., Weitzberg, E., 1999. Endothelin-A-receptor antagonism attenuates pulmonary hypertension in porcine endotoxin shock. Eur. Respir. J. 13, 145–151.

Wang, Q.D., Uriuda, Y., Pernow, J., Hemsen, A., Sjoquist, P.O., Ryden, L., 1995. Myocardial release of endothelin (ET) and enhanced ET_A receptor-mediated coronary vasoconstriction after coronary thrombosis and thrombolysis in pigs. J. Cardiovasc. Pharmacol. 26, 770–776.

Weitzberg, E., Lundberg, J.M., Rudehill, A., 1991. Elevated plasma levels of endothelin in patients with sepsis syndrome. Circ. Shock 33, 222–227.

Weitzberg, E., Rudehill, A., Lundberg, J.M., 1993. Nitric oxide inhalation attenuates pulmonary hypertension and improves gas exchange in endotoxine shock. Eur. J. Pharm. Sci. 233, 85–94.

Weitzberg, E., Lundberg, J.M., Rudehill, A., 1995. Inhibitory effects of diclofenac on the endotoxin shock response in relation to endothelin turnover in the pig. Acta Anaesthesiol. Scand. 39, 50–59.

Weitzberg, E., Hemsen, A., Rudehill, A., Modin, A., Wanecek, M., Lundberg, J.M., 1996. Bosentan-improved cardiopulmonary vascular performance and increased plasma levels of endothelin-1 in porcine endotoxin shock. Br. J. Pharm. 118, 617–626.

Vincent, J.L., 1998. Cardiovascular alterations in septic shock. J. Antimicriob. Chemother. 41, 9–15.

Vlessis, A., Goldman, R., Trunkey, D., 1995. New concepts in the pathophysiology of oxygen metabolism during sepsis. Br. J. Surg. 82, 870–876.