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Effects of moderate hypoxia, hypercapnia and acidosis on haemodynamic changes induced by endothelin-1 in the pithed rat

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1 Pithed rats were respired at a fixed rate of 54 cycles min^{-1} and with a ventilation volume of either 20 (control) or 10 ml kg^{-1}. In these two preparations, the dose-response relationships for the systemic blood pressure responses to endothelin-1, administered i.v., were examined. Also, cardiac output, its distribution, tissue blood flows and vascular resistances were determined at both respiratory volumes in pithed rats given saline or during pressor responses to endothelin-1 (750 ng, i.v.). Finally, a comparison was made of the pressor responses to endothelin-1 in the blood perfused superior mesenteric arterial bed of pithed rats respired at 10 or 20 ml kg^{-1}.

2 In control rats the systemic blood pressure responses to i.v. endothelin-1 were biphasic with an initial, transient (30 s) decrease in blood pressure followed by a well sustained pressor response. These responses were dose-dependent (the ED_{50} for the pressor response being 0.27 ± 0.04 μg). The pressor effect of endothelin-1 was due to an increase in total peripheral resistance with no change in heart rate or cardiac output. This increased total peripheral resistance was due to vasoconstriction of the spleen, stomach, large intestine, small intestine and the pancreas/mesentery (in which it was most severe). Endothelin-1 also increased blood flow through the heart, lungs, liver, epididimides, fat and skin through redistribution of cardiac output to these vascular beds.

3 At the lower ventilation volume there was moderate acidosis, hypoxia and hypercapnia relative to those rats respired at 20 ml kg^{-1}. With respiration at 10 ml kg^{-1}, the pressor response to endothelin-1 was not sustained and, after oscillations in both blood pressure and heart rate, death occurred 15–20 min after administration. The pressor effect resulted from increases in cardiac output (due to increased stroke volume) and total peripheral resistance: the latter was caused by vasoconstriction in the stomach, small intestine, large intestine and pancreas/mesentery. Endothelin-1 increased blood flow through the heart, lungs, liver, kidneys, testes, fat and skin due to either an increase in cardiac output, redistribution of cardiac output or both.

4 Endothelin-1 induced dose-dependent pressor responses in the mesenteric bed *in situ*. At the lower ventilation volume the potency of endothelin-1 in this vascular bed was increased approximately two fold with the ED_{50} being 68 ± 7 pmol compared to 113 ± 15 pmol in the rats respired at 20 ml kg^{-1}.

5 This study indicates that, in normoxic control pithed rats, the pressor response to endothelin-1 was due largely to vasoconstriction of the splanchnic vascular bed. In rats with moderate hypoxia, hypercapnia and acidosis, the pressor response was due to vasoconstriction of the gastrointestinal tract as well as an increase in cardiac output. Endothelin-1 induced profound vasoconstriction in the mesenteric bed of the pithed rat both *in vivo* and *in situ*. The potency of endothelin-1 on this bed *in situ* was doubled by lowering the ventilation volume. An increase in cardiac contractility and severe gastrointestinal vasoconstriction may be the initial events leading to the eventual toxic effect of endothelin-1 in the hypoxic pithed rat.

Introduction

It has been known for some time that hypoxic conditions potentiate vasoconstrictor responses to noradrenaline (Detar & Gellai, 1971). When it was found that pig pulmonary artery strips *in vitro* responded to hypoxia with a contraction that was reduced upon removal of the endothelium (Holden & McCall, 1984), it was suggested that the endothe-

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lium may release a contractile factor. Previously, it had been demonstrated that the anoxic facilitation of the canine arterial responsiveness to noradrenaline was endothelium-dependent (De Mey & Vanhoutte, 1982; 1983). Rubanyi & Vanhoutte (1984, 1985) demonstrated that hypoxia stimulates the release of a diffusible contractile factor from the canine coronary artery whilst Iqbal & Vanhoutte (1988) showed its activity was dependent upon extracellular calcium.

Other evidence that endothelial cells might mediate vasoconstriction has come from studies of cultured cells. Medium conditioned by endothelial cells was shown to contain a polypeptide which was a potent vasoconstrictor of coronary arteries and the contractions produced by this factor were found to be sensitive to calcium channel blocking agents (Hickey *et al.*, 1985; Gillespie *et al.*, 1986). Very recently, the sequence of endothelin-1, a vasoactive peptide released from endothelial cells in culture, has been determined and the vasoconstrictor activity of this peptide also shown to be dependent on the presence of extracellular calcium (Yanagisawa *et al.*, 1988). This has led to speculation that endothelin-1 may be the endothelium-derived vasoconstrictor which is wholly, or in part, responsible for the vasoconstriction and the potentiation of vascular responses to noradrenergic stimulation that occur in anoxia or hypoxia.

In the present study we have investigated the effect of a lower ventilation volume, which induces a mild hypoxia, hypercapnia and acidosis, on the systemic pressor response to endothelin-1 in the pithed rat. We have also determined the effects of endothelin-1, in both normoxic and hypoxic conditions, on cardiac output, cardiac output distribution, regional blood flows and regional vascular resistances in the pithed rat. Preliminary results from this study were presented to the Spring 1989 Meeting of the British Pharmacological Society (MacLean & Hiley, 1989).

Methods

Male Wistar rats (250–330 g; Bantin & Kingman Ltd, Hull) were pithed under halothane anaesthesia with a 16 gauge steel needle. Immediately after being pithed, the rats were respired with air through a tracheal cannula by means of a respiratory pump (BioScience, Sheerness, Kent) operating at 54 cycles min^{-1}. Body temperature was monitored by means of a rectal probe and maintained at 37°C by means of a homeothermic blanket system (BioScience, Sheerness, Kent).

Determination of dose-response curves

After pithing, the right carotid artery, left jugular vein and the right femoral artery of the rats were cannulated respectively for recording arterial blood pressure (in all animals blood pressure was recorded by means of a Bell & Howell type 4-422-0001 pressure transducer connected to a Grass Model 7D polygraph), for drug administration and for removal of the arterial blood samples used for determination of arterial blood gases and pH (see below). The rats were ventilated at a volume of 20 ml kg^{-1} and a dose-response curve to endothelin-1 constructed by giving bolus injections of the peptide (0.01–1 μg in 0.1–0.5 ml saline). The blood pressure was allowed to return to baseline between each dose and the doses of endothelin-1 were given in random order. The ED_{50} for endothelin-1 was calculated for each rat and the results are given as the mean ± s.e.mean. It was not possible to construct dose-response curves to endothelin-1 in rats ventilated at 10 ml kg^{-1}.

Determination of cardiac output, its distribution, regional blood flow and regional vascular resistance

Rats were pithed under halothane anaesthesia and were artificially respired with a volume of either 20 ml kg^{-1} (control) or 10 ml kg^{-1}. The right femoral artery was cannulated to measure systemic arterial blood pressure and heart rate was determined from the systemic blood pressure trace by a Grass 7P44 cardiotachograph. The left femoral artery was also cannulated and connected to a Perfusor IV pump (Braun, Melsungen, F.R.G.) for the withdrawal of blood. With the aid of pressure monitoring, a cannula was passed down the right common carotid artery into the left ventricle. Either endothelin-1 (750 ng; Novabiochem, Läufelfingen, Switzerland) in 0.3 ml saline, or 0.3 ml saline was administered through a cannula previously placed in the left external jugular vein. When a sustained blood pressure response had been obtained, 60,000–80,000 ^{113}Sn labelled microspheres (15 ± 3 mm; NEN, Boston, MA, U.S.A.), suspended by ultrasonication in saline containing 0.01% Tween 80, were injected into the ventricle over 20 s. Blood was withdrawn at 0.5 ml min^{-1} from the left femoral artery during, and for 70 s after, the microsphere injection. The circulation was stopped with an air embolism and the organs dissected out, weighed and placed in scintillation vials for counting in a Packard Autogamma 500 γ-scintillation counter.

The number of counts in the blood sample was also determined in order that cardiac output, tissue blood flow and organ vascular resistances could be measured as described by McDevitt & Nies (1976) and by us previously (MacLean & Hiley, 1988a,b). All results are given as the mean ± s.e.mean. Statistically significant differences between groups were assessed by analysis of variance followed by the least

significant difference procedure (Snedecor & Cochran, 1980).

Determination of dose-response curves in the blood perfused superior mesenteric arterial bed preparation of the pithed rat

Rats were anaesthetized with sodium thiopentone (Trapanal; BYK Gulden, Konstanz, F.R.G.), 120 mg kg^{-1} i.p., pithed and respired with a volume of either 20 ml kg^{-1} (control) or 10 ml kg^{-1} as described above. The left jugular vein was cannulated for the administration of drugs and 0.9% saline at 6 ml h^{-1} (to prevent volume depletion). The right common carotid artery was cannulated to record systemic blood pressure and heart rate as before. The preparation used for *in situ* blood perfusion of the superior mesenteric bed was a modification of the method of Jackson & Campbell (1980), which we have previously described in detail (Hiley *et al.*, 1985; Randall & Hiley, 1988; Randall *et al.*, 1989).

Endothelin-1 was administered into the extracorporeal circuit in 0.9% NaCl and in volumes of 100 μl or less. Dose-response relationships were determined by administration of doses in random order and the perfusion pressure allowed to return to baseline levels (5–20 min) before administration of the subsequent dose. EC_{50} values were calculated for each animal and final values are given as the mean ± s.e.mean.

Blood gases and pH were analysed in all animals by removing 125 μl blood samples from the extracorporeal circuit immediately before the first administration of endothelin-1 or saline. These samples were subsequently analysed with a Corning 166 micro blood gas analyser.

Results

Effect of lowering ventilation volume on blood Po_2, Pco_2 *and pH*

In 12 animals, lowering the ventilation volume from 20 to 10 ml kg^{-1} at 54 cycles min^{-1} induced moderate blood acidosis, hypoxia and hypercapnia. Blood pH was reduced from 7.44 ± 0.26 to 7.22 ± 0.12, blood Po_2 (mmHg) was reduced from 83.1 ± 2.4 to 59.8 ± 2.8 and blood Pco_2 (mmHg) was increased from 38.7 ± 1.9 to 61.4 ± 3.5. ($P < 0.001$ for each change, Student's paired t test).

Dose-response curves for the changes in mean arterial pressure induced by endothelin-1 in pithed rats ventilated at 20 ml kg^{-1} and 10 ml kg^{-1}

In rats respired at 20 ml kg^{-1}, endothelin-1 (25 ng–1 μg) induced biphasic changes in arterial blood pressure as shown in Figure 1; there was an initial, transient depressor response (lasting approximately 30 s) which was followed by a well-sustained pressor response (lasting 30 min–1 h). It can be seen from Figure 2 that the pressor responses to endothelin-1 were dose-dependent with an ED_{50} of 0.27 ± 0.04 μg. The initial depressor response was not clearly dose-dependent and the ED_{50} was therefore not calculated.

We have previously shown that even very low doses of endothelin-1 (10–30 ng) become toxic in rats in which the ventilation volume is 10 ml kg^{-1} (MacLean & Hiley, 1989) and for this reason a dose-response curve for endothelin-1 could not be constructed using this artificial respiratory volume. Figure 3 shows the response obtained to 100 ng endothelin-1 administered to a pithed rat respired at 10 ml kg^{-1}. Whilst all doses up to 1 μg were well tolerated in the normoxic state, it may be seen that, at the lower ventilation volume, a single dose of endothelin-1 induced a pressor response which was not maintained and which was followed by fluctuations in blood pressure and heart rate; death invariably occurred 15–20 min after endothelin-1 administration. Figure 3 also shows that the transient depressor response to endothelin-1 is absent in the hypoxic rat.

Effect of endothelin-1 on systemic haemodynamics

Table 1 shows the effect of endothelin-1 on haemodynamics at ventilation volumes of both 20 and 10 ml kg^{-1}. The lower ventilation volume in itself produced a basal mean arterial blood pressure which

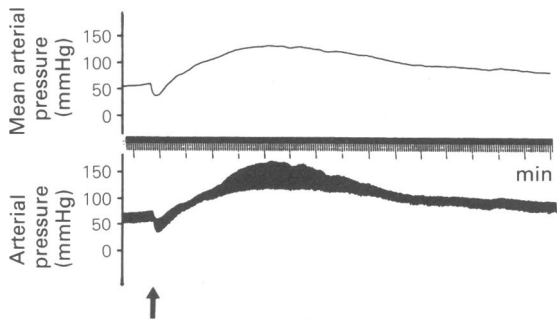

Figure 1 Experimental trace of blood pressure changes occurring after a single i.v. bolus injection of endothelin (750 ng) in a pithed rat respired with air at a ventilation volume of 20 ml kg^{-1}. The uppermost trace is the mean arterial pressure and the lowest trace shows instantaneous blood pressure; the middle trace shows timing marks with the distance between the largest marks indicating 1 min. The biphasic blood pressure response to endothelin may be seen clearly. The arrow indicates the point of endothelin administration.

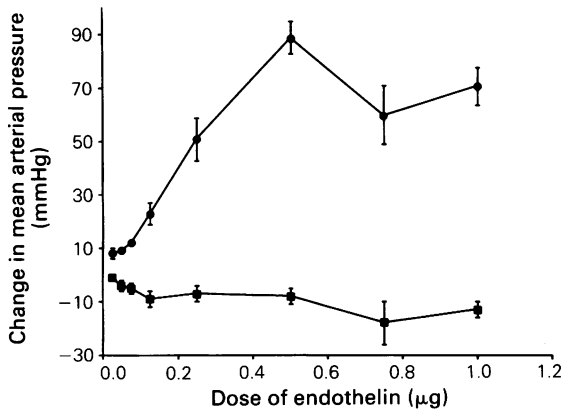

Figure 2 Blood pressure responses to endothelin in the pithed rat respired with air at a ventilation volume of $20\,ml\,kg^{-1}$ ($n = 5$). (■) The dose-response relationship for the initial, transient decrease in blood pressure to endothelin and (●) the dose-response relationship for the prolonged secondary pressor response to endothelin. The values are given as means with the vertical lines representing s.e.mean.

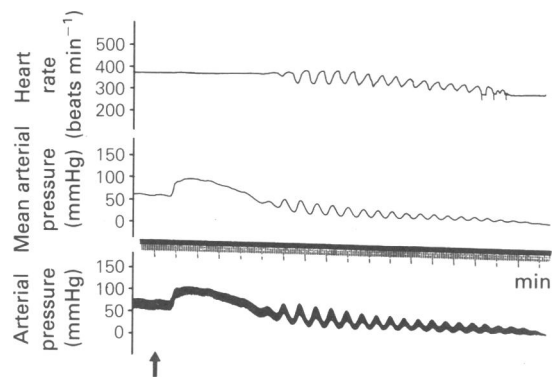

Figure 3 Experimental trace showing the blood pressure and heart rate responses to a single i.v. bolus injection of endothelin (100 ng) in a pithed rat respired with air at a ventilation volume of $10\,ml\,kg^{-1}$. From top to bottom the traces are: heart rate, mean blood pressure, timing scale with the largest marks indicating 1 min, and instantaneous blood pressure. The arrow indicates the point of endothelin administration.

was lower by 27% than in the rats respired at $20\,ml\,kg^{-1}$ (which will subsequently be referred to as normoxic rats). This difference was largely due to a lower pulse pressure since the basal diastolic pressures were not significantly different at the two respiratory volumes. In the normoxic rats, 750 ng endothelin-1 produced 51% and 61% increases in mean arterial and diastolic blood pressures, respectively, relative to the basal pressures observed before endothelin-1 administration. In the rats ventilated at $10\,ml\,kg^{-1}$ (subsequently referred to as hypoxic rats), the increases in mean arterial and diastolic blood pressures induced by 750 ng endothelin-1 were very similar in absolute terms to those occurring in the normoxic animals but, because of the lower basal mean arterial pressure, the increase in this in percentage terms, at 63%, was somewhat larger than in the controls. The diastolic blood pressure in the hypoxic animals was increased by 58% over the basal value.

In the normoxic rats there were no significant dif-

Table 1 The effects of endothelin-1 on haemodynamics in pithed rats ventilated with air at a rate of $54\,min^{-1}$ and a volume of either 20 or $10\,ml\,kg^{-1}$

	Saline ($20\,ml\,kg^{-1}$)	*Endothelin-1* ($20\,ml\,kg^{-1}$)	*Saline* ($10\,ml\,kg^{-1}$)	*Endothelin-1* ($10\,ml\,kg^{-1}$)
Basal mean arterial pressure (mmHg)	45 ± 2	41 ± 2	33 ± 3†	35 ± 3
Change in mean arterial pressure	−2 ± 1	21 ± 3***	−1 ± 1	22 ± 3***
Basal diastolic pressure (mmHg)	35 ± 2	33 ± 2	31 ± 3	31 ± 2
Change in diastolic pressure (mmHg)	−2 ± 1	20 ± 2***	−1 ± 1	18 ± 3***
Basal heart rate (beats min^{-1})	295 ± 15	325 ± 16	281 ± 8	284 ± 12
Change in heart rate (beats min^{-1})	1 ± 3	0 ± 7	−3 ± 1	−1 ± 3
Cardiac index (ml min^{-1} 100 g b.wt)	13.1 ± 0.9	13.8 ± 0.8	11.2 ± 1.0	14.8 ± 0.7*
Stroke volume (μl)	120 ± 7	111 ± 7	107 ± 13	151 ± 11*
TPR (mmHg ml^{-1} min 100 g b.wt)	3.3 ± 0.3	4.6 ± 0.3***	2.8 ± 0.3	4.0 ± 0.3**

For all groups, $n = 8$. Basal heart rate, diastolic and mean blood pressure values are those before administration of saline or endothelin-1. TPR = total peripheral resistance. The changes in blood pressure and heart rate are given as the differences between the values immediately before saline or endothelin-1 injection and those at the mid-point of the microsphere injection. Statistical differences were assessed by analysis of variance: between the two endothelin groups and their respective saline controls; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$: between the saline ($20\,ml\,kg^{-1}$) group and the saline ($10\,ml\,kg^{-1}$) group; † $P < 0.05$.

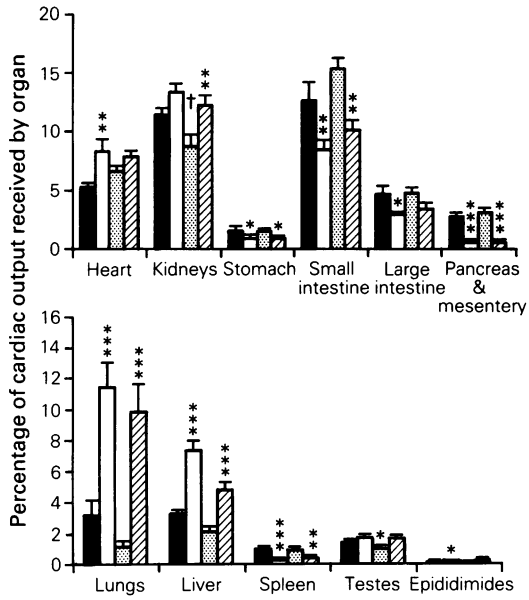

Figure 4 Effect of endothelin on the fractional distribution of cardiac output in pithed rats respired with air at either $20\,ml\,kg^{-1}$ or $10\,ml\,kg^{-1}$. The solid columns represent the results from rats given saline and ventilated at $20\,ml\,kg^{-1}$; the open columns are data from rats given 750 ng endothelin i.v. and ventilated at $20\,ml\,kg^{-1}$; the stippled columns are the data from rats given saline and ventilated at $10\,ml\,kg^{-1}$; and the hatched columns are the results from rats given 750 ng endothelin i.v. and ventilated at $10\,ml\,kg^{-1}$. $n = 8$ for all groups. Statistical differences were assessed by analysis of variance followed by the least significant difference procedure: between the endothelin groups and their respective saline controls; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$: between the saline group respired at $20\,ml\,kg^{-1}$ and the same group respired at $10\,ml\,kg^{-1}$; † $P < 0.05$.

ferences between the saline and endothelin-1 groups with respect to cardiac output, stroke volume or heart rate; the greater blood pressure after endothelin-1 was due to a 39% increase in total peripheral resistance. On the other hand, the hypoxic rats showed a 43% greater total peripheral resistance and a 32% greater cardiac index in the endothelin-1 group than in the saline group. The greater cardiac index was due to a 41% greater stroke volume since endothelin-1 had no effect on heart rate.

Effect of endothelin-1 on cardiac output distribution

Figure 4 shows that, in the normoxic animals, the endothelin-1 group showed fractions of cardiac output passing to the heart, lungs, liver and epididimides that were greater by 58%, 262%, 128% and 57% respectively relative to those in the saline group. There were, however, lesser proportions of the cardiac output passing to the spleen, stomach, small intestine, large intestine and pancreas/mesentery (by 73%, 43%, 34%, 39% and 79%, respectively) in the endothelin-1 relative to the saline group. Figure 4 also shows that, in the hypoxic saline group, the proportion of the cardiac output passing to the renal vascular bed was lower by 23% than in the equivalent normoxic group. Also, it may be seen that, in the hypoxic state, the endothelin-1 group showed, relative to the saline group, greater proportions of the cardiac output passing to the lungs (greater by 728%), liver (125%), kidneys (40%) and testes (57%) but lower proportions reached the spleen (lower by 52% than the saline group), stomach (45%), small intestine (34%) and pancreas/mesentery (81%).

Effects of endothelin-1 on regional blood flow

Table 2 shows that, in the normoxic animals, the endothelin-1 group had greater blood flows, relative to the saline group, through the heart, lungs, liver, fat, epididimides and skin (of 83%, 258%, 135%, 138%, 61% and 58% respectively) whilst there were lower flows through the spleen (less by 71%), small intestine (34%) and pancreas/mesentery (74%). Table 2 also shows that, in the hypoxic groups, the animals receiving endothelin-1 had, relative to the saline group, greater blood flows through the heart (45%), lungs (516%), liver (130%), kidneys (65%), testes (76%), fat (186%) and skin (107%) whilst blood flow through the pancreas/mesentery was decreased by 71% after endothelin.

Effects of endothelin-1 on regional vascular resistances

In the normoxic animals, vascular resistances in the splenic, stomach, small intestinal, large intestinal and mesenteric beds were 552%, 159%, 93%, 97% and 569% respectively greater in the endothelin-1 than in the saline group (Table 3). Coronary vascular resistance was 40% lower, and pulmonary vascular resistance greater by 149%, in the saline hypoxic group relative to the saline normoxic group. In the hypoxic group of rats given endothelin-1, coronary vascular resistance was still 26% lower than in the normoxic saline animals ($P < 0.05$). Table 3 also shows that, in the hypoxic animals, pulmonary and hepatic vascular resistances were lower by 88% and 43%, respectively, in the endothelin-1 group relative to the saline group but vascular resistances in the gastrointestinal tract were all significantly greater in the hypoxic, endothelin-1 group than in the hypoxic, saline group; the change for the stomach was 191% and those for the small intestine, large intestine and

Table 2 The effects of endothelin or saline on organ blood flow (ml min^{-1} g^{-1} organ wt) in pithed rats ventilated with air at a rate of 54 min^{-1} and a volume of either 20 ml kg^{-1} or 10 ml kg^{-1}

Organ	*Saline* (20 ml kg^{-1})	*Endothelin-1* (20 ml kg^{-1})	*Saline* (10 ml kg^{-1})	*Endothelin-1* (10 ml kg^{-1})
Heart	1.72 ± 0.11	3.14 ± 0.43**	2.19 ± 0.19	3.17 ± 0.25*
Lungs	0.92 ± 0.28	3.29 ± 0.51***	0.53 ± 0.21	3.26 ± 0.58***
Liver	0.091 ± 0.009	0.214 ± 0.022***	0.07 ± 0.017	0.161 ± 0.018***
Spleen	0.454 ± 0.063	0.132 ± 0.023**	0.385 ± 0.099	0.285 ± 0.046
Kidneys	1.85 ± 0.09	2.26 ± 0.08	1.48 ± 0.28	2.44 ± 0.23**
Testes	0.19 ± 0.013	0.231 ± 0.021	0.154 ± 0.024	0.271 ± 0.017**
Fat	0.026 ± 0.007	0.062 ± 0.013**	0.014 ± 0.004	0.040 ± 0.007*
Epididimides	0.082 ± 0.012	0.132 ± 0.012*	0.112 ± 0.011	0.13 ± 0.017
Skeletal muscle	0.094 ± 0.012	0.107 ± 0.024	0.081 ± 0.014	0.101 ± 0.018
Skin	0.073 ± 0.008	0.115 ± 0.017*	0.046 ± 0.011	0.095 ± 0.01**
Stomach	0.38 ± 0.055	0.226 ± 0.044	0.322 ± 0.045	0.251 ± 0.051
Small intestine	0.983 ± 0.149	0.646 ± 0.04*	0.966 ± 0.146	0.881 ± 0.064
Large intestine	0.691 ± 0.094	0.453 ± 0.051	0.674 ± 0.123	0.59 ± 0.074
Pancreas and mesentery	0.329 ± 0.031	0.084 ± 0.018***	0.31 ± 0.066	0.091 ± 0.016***

For all groups, $n = 8$. Statistical differences between the two endothelin groups and their respective saline controls were assessed by analysis of variance: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

pancreas/mesentery were respectively 94%, 108% and 389%.

Effect of lowering ventilation volume on the pressor responses to endothelin-1 in the blood perfused superior mesenteric bed of the pithed rat

In the rats used in this part of the study, there were no significant differences in mean arterial blood pressures or heart rates between the rats artificially respired at the two volumes; mean arterial blood pressure for the 20 ml kg^{-1} animals was 41 ± 4 mmHg and heart rate was 361 ± 21 beats min^{-1}, whilst for the hypoxic animals the respective values were 49 ± 4 mmHg and 353 ± 16 beats min^{-1}. Basal mesenteric perfusion pressure was, however, significantly lower ($P < 0.05$) in the hypoxic animals (49.1 ± 3.5 mmHg) when compared to the controls (65.0 ± 5.8). Figure 5 illustrates the log dose-response relationships for the pressor effects of endothelin-1 in the mesenteric bed of pithed rats ventilated at either 20 or 10 ml kg^{-1}. The curve was

Table 3 The effects of endothelin-1 or saline on organ vascular resistances (mmHg ml^{-1} min, g) in pithed rats ventilated at a rate of 54 min^{-1} and with a volume of 20 ml kg^{-1} or 10 ml kg^{-1}

Organ	*Saline* (20 ml kg^{-1})	*Endothelin-1* (20 ml kg^{-1})	*Saline* (10 ml kg^{-1})	*Endothelin-1* (10 ml kg^{-1})
Heart	25.8 ± 2.6	22.2 ± 2.5	15.5 ± 1.6†	19.2 ± 2.1
Lungs	69.9 ± 13.0	23.4 ± 4.4	174 ± 68†	21.0 ± 3.1*
Liver	490 ± 39	318 ± 37	654 ± 146	373 ± 18*
Spleen	109 ± 20	711 ± 228**	149 ± 52	251 ± 48
Kidneys	23.7 ± 2.29	27.8 ± 0.98	24.8 ± 2.7	24.8 ± 2.2
Testes	238 ± 34	286 ± 26	232 ± 25	216 ± 15
Fat	4165 ± 2073	1452 ± 340	3523 ± 737	1872 ± 388
Epididimides	603 ± 105	498 ± 42	301 ± 25	505 ± 81
Skeletal muscle	535 ± 99	1033 ± 341	454 ± 66	976 ± 371
Skin	619 ± 61	653 ± 110	941 ± 171	674 ± 106
Stomach	133 ± 25	344 ± 55**	110 ± 13	320 ± 75**
Small intestine	51.4 ± 9.4	99.2 ± 6.3***	35.7 ± 2.6	69.3 ± 7.9**
Large intestine	76.2 ± 17.6	150 ± 16**	54.7 ± 6.8	114 ± 19*
Pancreas and mesentery	143 ± 22	956 ± 147***	157 ± 51	767 ± 122***

For all groups, $n = 8$. Statistical differences were assessed by analysis of variance: between the two endothelin groups and their respective saline controls; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$: between the saline (20 ml kg^{-1}) group and the saline (10 ml kg^{-1}) group; † $P < 0.05$.

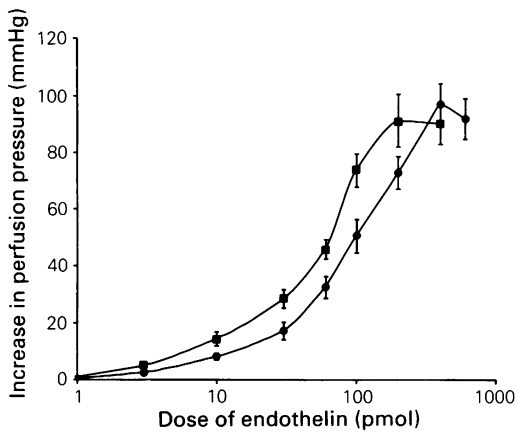

Figure 5 Pressor effects of endothelin in the *in situ* blood perfused, mesenteric arterial bed of rats respired at a ventilation volume of either $20\,ml\,kg^{-1}$ (●) or $10\,ml\,kg^{-1}$ (■) and at a rate of $54\,min^{-1}$. The values are given as means with the vertical lines representing s.e.mean ($n = 6$ for both groups).

shifted significantly ($P < 0.05$) to the left in the hypoxic animals with the ED_{50} decreased from 113 ± 15 pmol (normoxic) to 68 ± 7 pmol (hypoxic). The maximum pressor responses were not significantly different at the two ventilation volumes (Figure 5).

Discussion

Changes in mean arterial pressure induced by endothelin-1 in pithed rat

The results presented in Figures 1 and 2 show that endothelin-1 induces dose-dependent changes in blood pressure which are biphasic in nature in pithed rats artificially respired with room air at $20\,ml\,kg^{-1}$. It has been shown that endothelin-1 releases endothelium-derived relaxing factor (EDRF) in the rat isolated perfused mesenteric arterial bed (Warner *et al.*, 1989) although not, apparently, from cultured bovine aortic endothelial cells (De Nucci *et al.*, 1988). Thus, the initial, transient decrease in blood pressure observed before the onset of the sustained pressor response may be due to regional vasodilatation resulting from release of endothelium-derived relaxant factor (EDRF). However, this transient depressor response is absent in pithed rats ventilated at $10\,ml\,kg^{-1}$ and, in these animals, endothelin-1 becomes toxic at very low doses (MacLean & Hiley, 1989). It has been suggested that anoxia-induced inhibition of endothelium-dependent relaxation may contribute to anoxic facilitation of arterial contractions (De May & Vanhoutte, 1983) and, if so, this may explain why, in hypoxic conditions, the initial transient response to endothelin-1 was absent.

Haemodynamic changes induced by endothelin-1 in pithed rats artificially respired with air at $20\,ml\,kg^{-1}$

The results of this study show that, in the normoxic rats, the sustained increases in mean arterial pressure and diastolic blood pressure induced by endothelin-1 were due to an increase in total peripheral resistance as the peptide, at the dose used here, had no effects on cardiac output, stroke volume or heart rate. The increase in diastolic blood pressure was 10% greater than that observed from mean arterial pressure, an observation also made in anaesthetized, chemically denervated rats by Yanagisawa *et al.* (1988).

In comparison to saline-treated animals, endothelin-1 caused an increase in the fractional distribution of cardiac output to the heart, inducing an increase in coronary blood flow. Despite previous findings that endothelin-1 is a potent coronary vasoconstrictor and inotrope (Yanagisawa *et al.*, 1988; Hu *et al.*, 1988), neither of these effects of endothelin-1 were observed in this study. The increase in the fraction of cardiac output passing to the heart was not due to coronary vasodilatation but rather to redistribution of blood away from other vascular beds in which there was vasoconstriction, that is primarily the splenic and gastrointestinal circulations. Similarly, endothelin-1 also caused a redistribution of cardiac output towards the lungs, liver and epididimides, inducing increases in blood flow in these organs, without producing decreases in their vascular resistances. Endothelin-1 had no effect on blood flow through the testes or the renal vascular bed in the normoxic animals, but caused increases in blood flow through the fat and skin vascular beds. As there was no significant change in vascular resistance of these beds, the increase in flow must be due to redistribution of cardiac output.

Endothelin-1 caused decreases in splenic, small intestinal and mesenteric blood flows by causing vasoconstriction in these vascular beds; this resulted in corresponding decreases in the proportions of cardiac output they received. Although blood flows in the gastric and large intestinal vascular beds did not decrease significantly, there were increases in vascular resistance in these regions and these produced reductions in the fractional distribution of cardiac output to these vascular beds. In this study, endothelin-1-induced vasoconstriction was most profound in the mesenteric bed. Endothelin-1-induced mesenteric vasoconstriction has previously been observed in the anaesthetized spontaneously hypertensive rat (Wright & Fozard, 1988) and in conscious freely moving rats (Han *et al.*, 1989). From the

present study, it is evident that, in the pithed rat, the increase in total peripheral resistance observed following administration of endothelin-1 was largely due to vasoconstriction in the splanchnic region. Previous studies by other workers have also demonstrated that endothelin-1, at a dose which induced a higher increase in systemic blood pressure than observed in this study, increased total peripheral resistance by increasing vascular resistance in the kidneys and skeletal muscle as well as in the gastrointestinal tract (Thomas *et al.*, 1989; Walder *et al.*, 1989).

Effects of endothelin-1 on haemodynamics in pithed rats ventilated with air at 10 ml kg^{-1}

At the lower ventilation volume there was a reduced basal mean arterial blood pressure relative to the normoxic rats. In the absence of a functional sympathetic nervous system, as is the case in the pithed rat, humoral mechanisms, such as increased circulating angiotensin II (de Jonge *et al.*, 1982), must be involved in the maintenance of blood pressure. It is evident from the present study that these compensatory mechanisms are compromised in the hypoxic pithed rat and this confirms our previous results (MacLean & Hiley, 1988b). Since there were no significant differences between the hypoxic animals and the normoxic ones with respect to cardiac index or total peripheral resistance, although both means were numerically lower in the hypoxic animals, it is not possible to determine which of these was responsible for this slightly lower basal diastolic blood pressure. However, previous studies have shown that the direct effect of hypercapnia and hypoxia on the myocardium is to depress cardiac contractility (Price & Helrich, 1955; Sonnenblick & Kirk, 1972) and thus it is possible that decreased cardiac efficiency may be contributing to this effect. It is interesting to note that, in these pithed rats, the hypoxia combined with the hypercapnia and acidosis also induced coronary vasodilatation.

In the hypoxic rats, the increases in mean arterial and diastolic blood pressures induced by endothelin-1 were the result of an increase in both total peripheral resistance and cardiac output, unlike the situation in the control rats where the increase in blood pressure was solely due to an increase in total peripheral resistance. The increased total peripheral resistance in the hypoxic animals given endothelin-1 was due to profound vasoconstriction of the gastrointestinal tract, whilst the increased cardiac index was the result of an increase in stroke volume as, at this dose, endothelin-1 had no effect on heart rate. In the pithed rat, an increase in cardiac stroke volume could be caused by an increase in cardiac contractility or an increase in venous return. Endothelin-1 has been shown to exert potent inotropic effects in the rat atria (Hu *et al.*, 1988) and also to be a more potent vasoconstrictor of veins than arteries (D'Orleans-Juste *et al.*, 1988). With the knowledge that hypoxia-induced vasoconstriction can be endothelium-dependent and that the Po_2 of venous blood is lower than that of arterial, there is evidence to suggest a role for endothelin-1 in venous constriction, and hence, in maintenance of venous return. Thus, the increased stroke volume observed after endothelin-1 administration in the hypoxic state could be due to the combined effects of both increased venous return and cardiac contractility.

When comparing the saline groups, the lower ventilation volume produced a lower calculated coronary vascular resistance than in the normoxic animals. However, for the hypoxic rats, the increased coronary blood flow observed after endothelin-1 administration would appear to be the result of the increase in cardiac output since there was no significant difference between the two groups of rats given endothelin-1.

Hypoxia, hypercapnia and acidosis have previously been shown to induce increases in pulmonary vascular resistance (Bergofsky *et al.*, 1962; Sykes *et al.*, 1972) and this is observed here, the lowering of ventilation volume inducing an increase in the vascular resistance of the lungs. There was an increase in blood flow through the lungs following endothelin-1 administration in the hypoxic rats and this was due to the combined effects of the increased cardiac index and the apparent decrease in vascular resistance observed in this vascular bed. It should be noted that the microspheres detected in the lungs represent not only those passing through the bronchial arteries, but also those passing through arteriovenous anastomoses and which are subsequently trapped in the lungs after passage along the great veins. Thus these-apparent effects of endothelin-1 on lung blood flow could be due to actions on either the anastomoses or the bronchial arteries.

In the hepatic artery also there was a greater blood flow in the hypoxic animals given endothelin-1 relative to the hypoxic control group given saline. Here, as in the lungs, there was a lower calculated vascular resistance after the peptide and, thus, the enhanced rate of perfusion would appear to be the result of this reduced resistance as well as the increased cardiac output. It is known that the two vascular inputs into the liver interact by, apparently, purely mechanical processes, such that increases or decreases in hepatic portal venous supply are at least in part compensated by changes in hepatic arterial inflow resistance (Sato *et al.*, 1977). Thus an increase in resistance to portal inflow results in a decrease in hepatic arterial resistance and the changes in hepatic arterial resistance to flow seen in this study may

reflect opposing effects in gastrointestinal vascular resistance.

The decrease in the distribution of cardiac output to the spleen following endothelin-1 administration in these hypoxic rats was presumably due to redistribution of the cardiac output to other vascular beds (e.g. the lungs and liver), as there was no change in splenic vascular resistance. In view of the increased cardiac index, this decrease in distribution to the spleen resulted in there being no net effect on splenic blood flow. The endothelin-induced increases in renal and testicular blood flows observed in these animals were due to the combined effects of the increase in cardiac index and the proportions passing to these vascular beds; since there was no change in either testicular or renal vascular resistances, then this redistribution in their favour must be the result of them being relatively less resistant to flow after endothelin-1 than the gastrointestinal tract.

Despite the greater vascular resistances in the stomach, large intestinal and small intestinal beds relative to the hypoxic saline controls, the increased cardiac index resulted in there being no net alteration in the blood flow through these organs. The most profound vasoconstriction observed with endothelin-1 in the hypoxic rats was, as for the normoxic rats, observed in the mesenteric bed. In this region there was a much lower blood flow than in the hypoxic saline controls since the redistribution of cardiac output away from this bed, resulting from the vasoconstriction induced by endothelin-1, was sufficient to overcome the effects of the increase in cardiac output.

Effect of a lower ventilation volume on the pressor responses to endothelin-1 in the blood perfused superior mesenteric bed of the pithed rat

Dose-dependent vasoconstriction of the superior mesenteric arterial bed by endothelin-1 has previously been formed both in isolated (De Nucci *et al.*, 1988; Hiley *et al.*, 1989) preparations and in similarly anaethetized intact rats in which the bed has been perfused *in situ* with blood (Hiley *et al.*, 1989). The present study confirms that dose-dependent increases in perfusion pressure also occur in the blood perfused mesenteric arterial bed of pithed rats. Hence, both in the intact circulation and in the *in situ* isolated blood perfused bed, endothelin-1 causes profound constriction in the mesenteric vasculature of the pithed rat.

The results also show that it is the potency of endothelin-1 on this vascular bed which changes when the rats are respired with the lower respiratory volume. Thus, in hypoxic, hypercapnic and acidotic conditions the peptide is twice as potent as in the normoxic group of animals and the maximum response is unchanged. This may be due to the inhibition by hypoxia of the release of EDRF by endothelin, but we have been unable to confirm this because we have found that, in similarly anaesthetized rats, removal of the endothelium with detergent in this preparation severely reduces the maximum response to endothelin-1, something that we have not observed for other vasoconstrictor agonists (Randall *et al.*, 1989). Other possibilities for this apparent increase in potency include changes in receptor number, affinity or efficiency of coupling to effector mechanisms.

Conclusions

In conclusion, the results of this study indicate that, in the normoxic pithed rat, the systemic pressor effect of endothelin-1 is mainly due to profound vasoconstriction of the splanchnic vascular beds. In the hypoxic rats, the pressor effect is due to a combination of an increase in gastrointestinal vasoconstriction and an increase in cardiac output. Endothelin-1 is very toxic in rats with moderate hypoxia, hypercapnia and acidosis with death occurring within 20 min of a low dose and being associated with disturbances of heart rate. The results presented here are consistent with this being due to an effect of endothelin-1 on cardiac contractility which eventually leads to deterioration of cardiac performance. The severe endothelin-induced gastrointestinal vasoconstriction, particularly that of the mesenteric bed, may contribute to the eventual cardiovascular collapse. It may be that any physiological role for endothelin release and activity are compromised in vascular disease states such as hypotension, hypertension and hypoxia. If so, it may play a role in phenomena like the acute renal failure observed in hypotension and hypoxia (Firth *et al.*, 1988), in myocardial ischaemic conditions and in splanchnic vasoconstriction associated with shock states.

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