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HAEMODYNAMIC AND RENAL TUBULAR EFFECTS OF LOW DOSES OF ENDOTHELIN IN ANAESTHETIZED RATS

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SUMMARY

1. Renal haemodynamic and tubular transport responses to low-dose infusions (1 and 10 ng kg^{-1} min^{-1}) of endothelin were investigated in anaesthetized rats.

2. Both doses caused transient increases in mean arterial blood pressure (17 ± 5 mmHg, $P < 0.05$ at 1 ng kg^{-1} min^{-1}) followed by sustained hypotension (-14 ± 5 mmHg, $P < 0.05$), reduced renal vascular resistance (−42%, $P < 0.05$) and increased renal plasma flow (46%, $P < 0.05$). Glomerular filtration rate was unchanged.

3. Each dose caused profound diuresis and natriuresis. At 1 ng kg^{-1} min^{-1} urine flow rate and fractional water excretion increased 5-fold and fractional sodium excretion 10-fold. Fractional potassium excretion and solute-free water clearance were unaltered.

4. End-proximal fluid delivery estimated by lithium clearance doubled ($P < 0.05$) and fractional proximal and distal sodium reabsorption decreased 10–20% ($P < 0.05$). Absolute proximal reabsorption also fell with the higher dose.

5. Hypotension and natriuresis persisted for 30 min after terminating infusions. Time-control animals showed no changes in haemodynamics or renal tubular transport.

6. It is concluded that endothelin, at low concentrations, causes renal vasodilatation with concomitant natriuresis due to reduced sodium transport in proximal and distal nephron segments.

INTRODUCTION

Endothelin is a potent vasoactive polypeptide recently isolated from the supernatant of cultured porcine aortic endothelial cells (Yanagisawa, Kurihara, Kimura, Tomobe, Kobayashi, Mitsui, Yazaki, Goto & Masaki, 1988). It is now believed that three isomers exist in all species and pharmacological studies have shown all endothelins to be extremely potent vasoconstrictors (Yanagisawa *et al.* 1988). *In vitro*, endothelin induces sustained contraction of vascular smooth muscle in a variety of blood vessels including coronary, basilar, aortic, femoral and mesenteric arteries from many species (Tomobe, Miyauchi, Saito, Yanagisawa, Kimura, Goto & Masaki, 1988; Yanagisawa *et al.* 1988; Cocks, Broughton, Dib,

Sudhir & Angus, 1989; Minkes, MacMillan, Bellan, Kerstein, McNamara & Kadowitz, 1989). *In vivo*, intravenous bolus injections of endothelin cause prolonged systemic hypertension in anaesthetized and chemically denervated rats (Yanagisawa *et al.* 1988). From these observations it has been suggested that endothelin may be involved in the control of systemic arterial blood pressure and regional blood flow and contribute to the pathogenesis of hypertension, vascular spasm (Yanagisawa *et al.* 1988) and acute renal failure (Firth, Ratcliffe, Raine & Ledingham, 1988).

Low doses of endothelin have, however, been reported to dilate rather than constrict systemic vascular vessels *in vitro* (Wright & Fozard, 1988; Minkes *et al.* 1989; Warner, de Nucci & Vane, 1989*a*), and to decrease arterial blood pressure *in vivo* (Lippton, Goff & Hyman, 1988; Minkes & Kodowitz, 1989; Minkes *et al.* 1989; Winquist, Bunting, Garsky, Lumma & Schofield, 1989*a*). The systemic response to endothelin therefore appears to be dose- and time-dependent.

In the rat kidney, specific high-affinity binding sites for endothelin have been localized to intrarenal arterial structures and glomeruli (Orita, Fujiwara, Ochi, Takama, Fukunaga & Yokoyama, 1989), inner medulla, vasa recta bundles and proximal convoluted tubules (Kohzuki, Johnston, Chai, Casley & Mendelsohn, 1989). Endothelin has been shown to stimulate mitogenesis and activate the phosphoinositide cascade in cultured rat glomerular mesangial cells (Simonson, Wann, Mene, Dubyak, Kester, Nakazato, Sedor & Dunn, 1989), and to inhibit Na^+–K^+-ATPase in the inner medullary collecting duct cells *in vitro* (Zeidel, Kone, Brady, Gullans & Brenner, 1989). However, studies of the global effects of endothelin on renal function have been inconclusive and controversial. High concentrations of endothelin decreased glomerular filtration rate and renal plasma flow in parallel in isolated perfused rat kidneys (Firth *et al.* 1988) and in anaesthetized rats (Hirata, Matsuoka, Kimura, Fukui, Hayakana, Suzuki, Sugimoto, Sugimoto, Yanagisawa & Masaki, 1989; Lopez-Farre, Montanes, Millas & Lopez-Novoa, 1989), and markedly reduced urinary sodium excretion in dogs (Goetz, Wang, Madwed, Zhu & Leadley, 1988; Miller, Refield & Burnett, 1989). These effects are consistent with the vasoconstrictor action of high doses of endothelin on isolated renal artery *in vitro* (Tomobe *et al.* 1988; Yanagisawa *et al.* 1988). However, a renal vasodilator response to low doses of endothelin has been demonstrated in anaesthetized cats (Lippton *et al.* 1988) and rats (Wright & Fozard, 1988). Since the plasma concentrations of immunoreactive endothelin detected in normal subjects (10–20 pg ml^{-1}, Suzuki, Matsumoto, Kitada, Yanagisawa, Miyauchi, Masaki & Fujino, 1989; Saito, Nakao, Yamada, Itoh, Mukoyama, Arai, Hosoda, Shirakami, Suga, Jougasaki, Ogawa & Imura, 1989) and hypertensive patients (30–70 pg ml^{-1}, Saito *et al.* 1989) are more than 100- to 1000-fold less than those required for direct systemic and renal vasoconstriction, it is reasoned that a dilator rather than constrictor action may underlie the physiological role of endothelin in the regulation of renal function.

The present experiments were undertaken to characterize the effects of intravenous infusion of low doses of endothelin on systemic arterial blood pressure, renal haemodynamics and tubular reabsorption of sodium and water in anaesthetized rats. Clearance methods were used to monitor renal plasma flow and glomerular filtration rate and the extent of proximal and distal tubular transport of sodium and water was estimated using lithium clearance (Thomsen, 1984).

METHODS

Dietary preparation and surgical procedures

Experiments were carried out on thirty-eight male Long–Evans rats (250–350 g). Rats were initially maintained on a standard laboratory diet containing 100 mmol sodium chloride per kilogram dry weight food and allowed free access to tap water. Two days before experimentation, all rats were caged individually and fed a wet-mash diet prepared by adding 15 mmol lithium chloride per kilogram to the dry standard diet. Dietary sodium was maintained at this level, thus avoiding any sodium-restriction which has been shown to enhance lithium reabsorption in distal nephron segments (Thomsen & Leyssac, 1986; Kirchner 1987). On the days of experiments, rats were anaesthetized with Inactin (110 mg (kg body wt)$^{-1}$, I.P.) and surgically prepared as described previously (Harris, Skinner & Zhuo, 1989).

Experimental protocol

Upon completion of surgical procedures, a priming dose (1 ml kg^{-1}) of isotonic saline containing 8% polyfructosan (Inutest, Laevosan-Gesellschaft, Linz, Donau) and 1% *p*-aminohippuric acid (PAH, Sigma) was administered. Separate sustaining infusions at 0·0375 ml min^{-1} of 8% polyfructosan with 1% *p*-aminohippuric acid in 0·9% NaCl, and 4 mM-lithium chloride in 0·9% NaCl, were commenced from two syringes and continued throughout the experiment. The total infusion rate of isotonic saline was 0·075 ml min^{-1}. A 2 h equilibration period was allowed before clearance measurements were made.

Immediately after the equilibration period, all rats were subjected to an identical protocol in which three consecutive 20 min urine collections were made during each of four 60 min periods. An arterial blood sample (250 μl) was collected from the carotid artery at the mid-point of each 60 min period. A small part of these blood samples was used to measure haematocrit while the remainder was centrifuged, and the plasma collected and stored at −20 °C for assay. The separated blood cells were resuspended in saline and injected slowly into the animal via a jugular vein catheter. At the end of the experiment both kidneys were removed, blotted and weighed. Rats were allocated to five groups.

Group 1 ($n = 8$): *time-control* (*vehicle infusion*). Rats in this group received only saline and clearance markers and no endothelin. Blood and urine collections were performed as for other groups.

Group 2 ($n = 8$): *endothelin infusion*. After the first 60 min period (control), porcine endothelin (Auspep, Australia) was added to the lithium infusion and delivered at a constant rate of 1 ng kg^{-1} min^{-1} over 90 min. Mean arterial blood pressure (MABP) was measured at 5 mins for the first 30 min of the endothelin period and then every 20 min during the remaining 60 min. Urine collections were made during three 20 min periods beginning 30 min after initiation of the endothelin infusion. This equilibration period ensured steady-state conditions and minimized the possibility of dead-space errors. During the second experimental period starting 30 min after completion of the first dose of endothelin, the higher dose of endothelin (10 ng kg^{-1} min^{-1}) was infused for 90 min. MABP was measured and urine samples collected as for the first period.

Group 3 ($n = 10$). Rats in this group were used for control experiments to determine the plasma concentration of atrial natriuretic factor (ANF). All rats were treated similarly to those in group 1, and received only saline and clearance markers but urine samples were not collected. An arterial blood sample (3 ml) was collected from the carotid artery at the end of the second experimental period. The blood was centrifuged, and plasma collected and stored at −70 °C for later immunoassay of plasma ANF concentration.

Group 4 ($n = 8$). Rats in this group were used to monitor the plasma concentration of ANF during infusion of endothelin. All rats were treated similarly to those in group 3 except that during the second experimental period endothelin was infused at the rate of 1 ng kg^{-1} min^{-1} for 90 min.

Group 5 ($n = 5$). Since the hypotensive and natriuretic response to infusion of endothelin in group 2 were unexpected, additional experiments were conducted using the protocol employed for group 2 to examine whether contamination of the endothelin might have contributed to the observed responses. Another brand of synthetic porcine endothelin (Peptide Institute, Osaka, Japan) was infused at 1 ng kg^{-1} min^{-1} for 90 min during the second experimental period. Urine and plasma samples were collected as in group 2.

Analytical methods and calculations

Urine flow rate was determined gravimetrically and arterial haematocrit measured by the microcapillary method. Sodium and potassium concentrations in plasma and urine samples were measured by flame photometry (Model IL943, Instrumentation Laboratories, Lexington, MA, USA) and lithium concentrations in plasma and urine by atomic absorption spectrophotometry (Model 901, GBC Scientific, Melbourne, Australia). Osmolalities of plasma and urine samples were determined using a vapour pressure osmometer (Wescor, Logan, UT, USA). Polyfructosan concentrations in plasma and urine were determined by the anthrone method (Fuhr, Kaczmerczyk & Kruttgen, 1955) and *p*-aminohippuric acid by the method of Smith, Finkelstein & Aliminosa (1945). Plasma concentration of atrial natriuretic factor was determined as described by Tsunoda, Hodsman, Sumithran & Johnson (1986).

The standard clearance equation was used to calculate the renal clearance of polyfructosan, *p*-aminohippuric acid and lithium which were taken as indices of glomerular filtration rate (GFR), effective renal plasma flow (ERPF) and end-proximal fluid delivery (C_{Li}) of the whole nephron population. Renal plasma flow (RPF) was then calculated assuming a value of 90% for the renal extraction of *p*-aminohippuric acid. Renal vascular resistance was calculated as mean arterial blood pressure divided by renal blood flow (RBF). Total absolute proximal reabsorption (APR) was derived as GFR-C_{Li}, fractional proximal reabsorption of sodium (FPR_{Na}) determined as fractional lithium reabsorption (FR_{Li}), and fractional distal reabsorption of sodium calculated as a fraction of end-proximal sodium delivery reabsorbed in the distal nephron segments. Osmolar clearance (C_{osm}) was calculated as $U_{osm}V/P_{osm}$ and solute-free water clearance (C_{H_2O}) as $V-C_{osm}$.

Statistical analysis

Renal function parameters from control and experimental periods were averaged from three 20 min consecutive clearance periods. All data are expressed as means ± S.E.M. Results within each experiment were analysed by one-way analysis of variance for repeated measurements of the same variable. The Newman–Keuls method was used to determine which mean values differed statistically from those obtained during control periods. Data from the corresponding periods between group 1 and group 2, between group 3 and group 4, and results between control and experimental periods in group 5 were compared using Student's unpaired *t* test. Differences were considered significant if $P < 0.05$.

RESULTS

Groups 1 and 2: time-control and endothelin infusion experiments

Effect of endothelin on haematocrit, plasma concentrations of electrolytes and clearance markers, and mean arterial blood pressure (MABP)

As shown in Table 1, there were no significant changes in arterial haematocrit or in the plasma concentrations of electrolytes and clearance markers throughout the measurement periods.

During infusion of endothelin at 1 ng kg^{-1} min^{-1}, MABP showed a consistent biphasic response (Fig. 1), increasing within 5 min of the beginning of infusion (10 ± 3 mmHg, $P < 0.05$) and reaching a maximum within 10 min (17 ± 6 mmHg, $P < 0.05$). However, the hypertensive component of the response was transient and blood pressure fell below control values 20 min after initiation of infusion (−8 ± 5 mmHg, $P < 0.05$) and remained low throughout the following 60 min of infusion (−15 ± 5 mmHg, $P < 0.05$). Neither hypertensive nor hypotensive phases were dose-related since responses of similar magnitude were observed with both doses (Fig. 2). Hypotension persisted during the recovery period (30–90 min after infusion of endothelin was stopped).

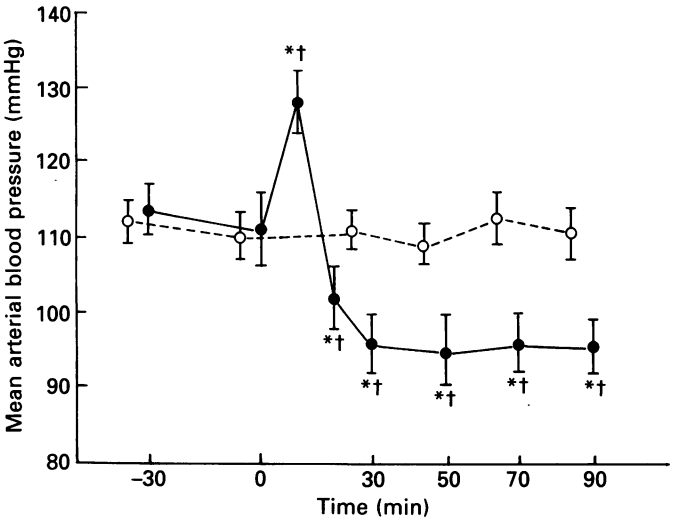

Fig. 1. Biphasic response of mean arterial blood pressure to low-dose infusion of endothelin (●, 1 ng kg^{-1} min^{-1}) in group 2 rats compared with saline time-controls (○) in group 1. * indicates significant difference from control within a group ($P < 0.05$). † indicates significant difference from corresponding periods between groups ($P < 0.05$). Statistical comparisons were performed by one-way analysis of variance for repeated measurements and the Newman–Keuls method within groups, and by unpaired t tests between groups.

TABLE 1. Effects of low doses of endothelin on arterial haematocrit (Hct) and plasma concentrations of electrolytes and clearance markers

		Endothelin		
Variables	Control	ET-1	ET-10	Recovery
Hct (%)	44·8±1·0	44·6±0·9	43·9±1·3	43·1±1·4
P_{Na} (mmol l^{-1})	143·2±0·6	143·2±0·5	143·8±0·7	142·1±1·4
P_K (mmol l^{-1})	3·63±0·2	3·50±0·1	3·40±0·1	3·30±0·2
P_{Li} (mmol l^{-1})	0·23±0·05	0·21±0·07	0·20±0·09	0·20±0·07
P_{In} (mmol l^{-1})	0·32±0·01	0·29±0·02	0·28±0·02	0·27±0·04
P_{PAH} (mg ml^{-1})	0·06±0·01	0·05±0·01	0·06±0·01	0·05±0·01

ET-1, 1 ng kg^{-1} min^{-1}. ET-10, 10 ng kg^{-1} min^{-1}. P_{Na}, plasma sodium concentration. P_K, plasma potassium concentration. P_{Li}, plasma lithium concentration. P_{In}, plasma polyfructosan concentration. P_{PAH}, plasma concentration of *p*-aminohippuric acid.

Effects of endothelin on urinary excretion of water, sodium and potassium, and osmolar and solute-free water clearances

With both doses, marked diuresis developed during the infusions (Fig. 3). Urine flow rate increased within 5 min and reached a steady state 30 min after the start of the infusion (14·6±1·5 μl min^{-1} during control to 71·4±14·4 μl min^{-1} with 1 ng kg^{-1} min^{-1}, $P < 0.05$, and to 72·3±4·2 μl min^{-1}, $P < 0.05$, with 10 ng kg^{-1} min^{-1}). Fractional water excretion (FE_{H_2O}) increased in parallel with urine flow rate (from a

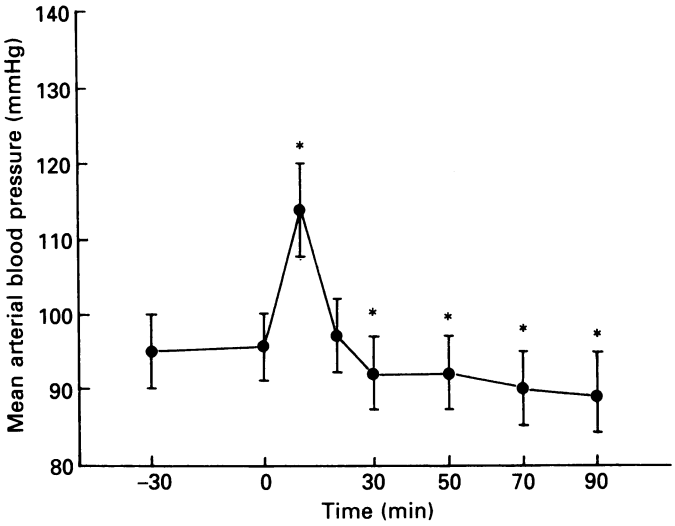

Fig. 2. Biphasic response of mean arterial blood pressure to higher-dose infusion of endothelin (10 ng kg^{-1} min^{-1}) in group 2 animals. The method and levels of statistical analysis are as in Fig. 1.

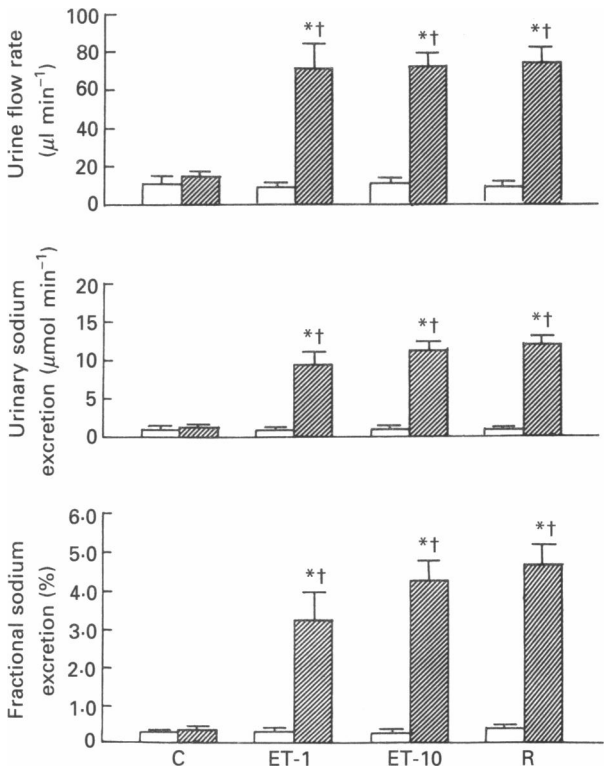

Fig. 3. Changes in urine flow rate, and absolute and fractional sodium excretion in rats receiving saline vehicle (□) and infusion of endothelin (▨). C, control period. ET-1, the first experimental period with infusion of endothelin at 1 ng kg^{-1} min^{-1}. ET-10, the second experimental period with infusion of endothelin at 10 ng kg^{-1} min^{-1}. R, recovery period. Symbols and levels of statistical significance are as in Fig. 1.

control value of $0{\cdot}82 \pm 0{\cdot}08$ % to $3{\cdot}65 \pm 0{\cdot}87$ % at 1 ng kg^{-1} min^{-1}, and to $3{\cdot}94 \pm 0{\cdot}39$ % at 10 ng kg^{-1} min^{-1}). Diuresis persisted during the recovery period ($V = 74{\cdot}5 \pm 7{\cdot}9$ μl min^{-1}, $P < 0{\cdot}05$; $FE_{H_2O} = 4{\cdot}05 \pm 0{\cdot}48$ %, $P < 0{\cdot}05$).

As shown in Fig. 3, endothelin induced marked natriuresis. Compared with control, urinary sodium excretion ($U_{Na}V$) increased more than 10-fold with each dose

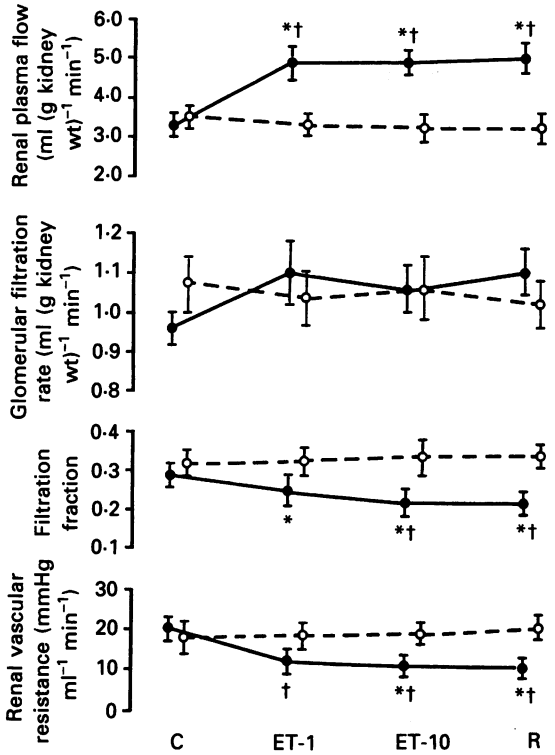

Fig. 4. Changes in renal haemodynamics in rats receiving saline vehicle (○) and infusion of endothelin (●). Abbreviations, symbols and levels of statistical significance are as in Fig. 3.

(from $0{\cdot}92 \pm 0{\cdot}10$ to either $9{\cdot}31 \pm 1{\cdot}95$ μmol min^{-1} or to $11{\cdot}29 \pm 0{\cdot}52$ μmol min^{-1} respectively, $P < 0{\cdot}05$). Fractional sodium excretion (FE_{Na}) increased similarly with each dose (from $0{\cdot}37 \pm 0{\cdot}07$ to $3{\cdot}34 \pm 0{\cdot}84$ % or to $4{\cdot}35 \pm 0{\cdot}44$ %, $P < 0{\cdot}05$). Natriuresis continued during the recovery period, and $U_{Na}V$ ($12{\cdot}20 \pm 0{\cdot}93$ μmol min^{-1}, $P < 0{\cdot}05$) and FE_{Na} ($4{\cdot}77 \pm 0{\cdot}50$ %, $P < 0{\cdot}05$) showed no tendency to return towards the control values.

Endothelin increased urinary potassium excretion (U_KV) from $1{\cdot}53 \pm 0{\cdot}16$ μmol min^{-1} during control to $2{\cdot}13 \pm 0{\cdot}15$ μmol min^{-1} ($P < 0{\cdot}05$) with the low dose, and to $1{\cdot}97 \pm 0{\cdot}10$ μmol min^{-1} ($P < 0{\cdot}05$) with the high dose and remained elevated during the recovery period ($1{\cdot}96 \pm 0{\cdot}23$ μmol min^{-1}, $P < 0{\cdot}05$). However, fractional potassium excretion (FE_K) was unaltered throughout the experiment.

Osmolar clearance doubled with each dose (from $57{\cdot}3 \pm 3{\cdot}4$ to $112{\cdot}2 \pm 13{\cdot}6$ osmol $(kg\ H_2O)^{-1}$, $P < 0{\cdot}05$, and to $121{\cdot}7 \pm 4{\cdot}5$ osmol $(kg\ H_2O)^{-1}$ respectively, $P < 0{\cdot}05$)

and was not diminished during the recovery period ($127{\cdot}9 \pm 12{\cdot}8$ osmol (kg H_2O)$^{-1}$, $P < 0{\cdot}05$). Nevertheless, endothelin had no significant effect on solute-free water clearance.

Effect of endothelin on renal haemodynamics

Figure 4 shows that endothelin caused marked renal vasodilatation. RPF increased from the control value of $3{\cdot}34 \pm 0{\cdot}22$ ml (g kidney wt)$^{-1}$ min^{-1} to $4{\cdot}90 \pm 0{\cdot}41$ (g kidney wt)$^{-1}$ min^{-1} with the low dose ($P < 0{\cdot}05$), and to $4{\cdot}91 \pm 0{\cdot}28$ ml (g kidney wt)$^{-1}$ min^{-1} with the higher dose ($P < 0{\cdot}05$). No dose dependence of the response was observed and the increased RPF persisted during the recovery period ($4{\cdot}96 \pm 0{\cdot}38$ ml (g kidney wt)$^{-1}$ min^{-1}, $P < 0{\cdot}05$). Consistent with the increases in renal blood flow despite systemic hypotension, calculated RVR was markedly reduced from control ($19{\cdot}8 \pm 1{\cdot}3$ to $11{\cdot}5 \pm 1{\cdot}1$ mmHg ml^{-1} min^{-1} or to $10{\cdot}9 \pm 0{\cdot}92$ mmHg ml^{-1} min^{-1}) with each dose ($P < 0{\cdot}05$) and did not return to control after endothelin infusion was stopped ($10{\cdot}4 \pm 0{\cdot}7$ mmHg ml^{-1} min^{-1}, $P < 0{\cdot}05$).

As illustrated in Fig. 4, GFR was not significantly altered from control ($0{\cdot}94 \pm 0{\cdot}04$ to $1{\cdot}16 \pm 0{\cdot}13$ ml (g kidney wt)$^{-1}$ min^{-1} or to $1{\cdot}06 \pm 0{\cdot}13$ ml (g kidney wt)$^{-1}$ min^{-1} with each dose, $P > 0{\cdot}050$ and remained unchanged during the recovery period ($1{\cdot}10 \pm 0{\cdot}10$ ml (g kidney wt)$^{-1}$ min^{-1}).

Since endothelin increased RPF but not GFR, FF decreased from a control value of $0{\cdot}29 \pm 0{\cdot}01$ to $0{\cdot}25 \pm 0{\cdot}04$ with the low dose ($P < 0{\cdot}05$) and to $0{\cdot}22 \pm 0{\cdot}03$ with the higher dose ($P < 0{\cdot}05$), and remained low 30 min after infusion of endothelin ceased ($0{\cdot}22 \pm 0{\cdot}03$, $P < 0{\cdot}05$).

Effect of endothelin on renal tubular reabsorption of sodium and water

Although filtered load was not significantly altered by endothelin, C_{Li} doubled with both the lower dose (from $0{\cdot}26 \pm 0{\cdot}03$ to $0{\cdot}48 \pm 0{\cdot}06$ ml (g kidney wt)$^{-1}$ min^{-1}, $P < 0{\cdot}05$) and the higher dose (to $0{\cdot}53 \pm 0{\cdot}06$ ml (g kidney wt)$^{-1}$ min^{-1}, $P < 0{\cdot}05$, Fig. 5). C_{Li} failed to return to control during the recovery period ($0{\cdot}48 \pm 0{\cdot}06$ ml (g kidney wt)$^{-1}$ min^{-1}, $P < 0{\cdot}05$).

FPR_{Na} was suppressed by endothelin from $72{\cdot}6 \pm 2{\cdot}3\%$ during control to $61{\cdot}6 \pm 4{\cdot}1\%$ and to $51{\cdot}8 \pm 4{\cdot}0\%$ ($P < 0{\cdot}05$) with each dose respectively. FPR_{Na} remained depressed for 30–90 min during the recovery period ($58{\cdot}9 \pm 4{\cdot}8\%$, $P < 0{\cdot}05$).

APR was not significantly altered with the low dose of endothelin ($0{\cdot}67 \pm 0{\cdot}04$ to $0{\cdot}65 \pm 0{\cdot}09$ ml (g kidney wt)$^{-1}$ min^{-1}) but decreased by 20% with the high dose ($0{\cdot}53 \pm 0{\cdot}09$ ml (g kidney wt)$^{-1}$ min^{-1}, $P < 0{\cdot}05$). However, during the recovery period, APR returned to a level that was not significantly different from control ($0{\cdot}62 \pm 0{\cdot}09$ ml (g kidney wt)$^{-1}$ min^{-1}, $P < 0{\cdot}05$).

Although endothelin markedly increased end-proximal delivery of solute and fluid, FDR_{Na} decreased significantly from $98{\cdot}6 \pm 0{\cdot}3\%$ during control to $93{\cdot}2 \pm 1{\cdot}4\%$ with the low dose ($P < 0{\cdot}05$), and to $91{\cdot}5 \pm 0{\cdot}8\%$ with the high dose ($P < 0{\cdot}05$). Suppression of FDR_{Na} persisted during the recovery period ($89{\cdot}3 \pm 1{\cdot}7\%$, $P < 0{\cdot}05$).

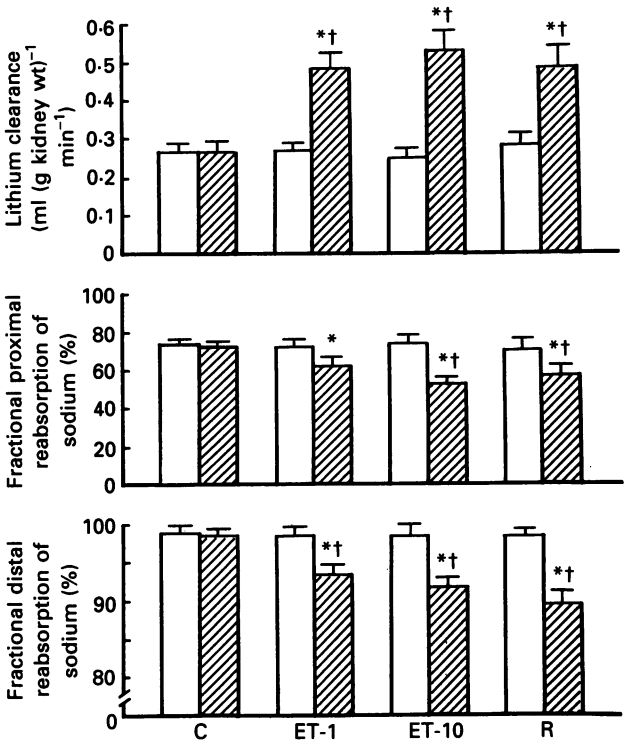

Fig. 5. Renal tubular transport responses to constant infusion of low doses of endothelin (▨) compared with saline vehicle (□). Abbreviations, symbols and levels of statistical significance are as in Fig. 3.

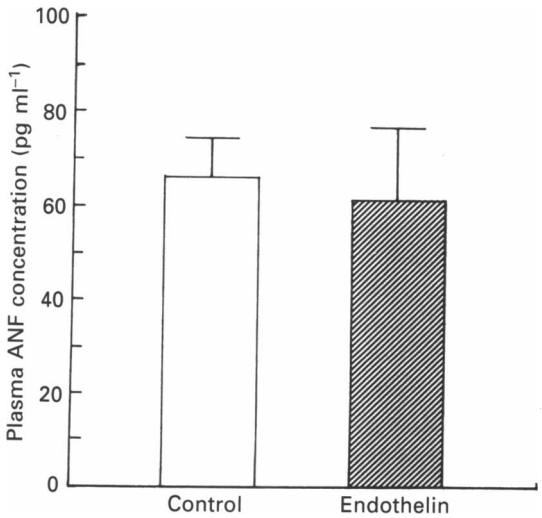

Fig. 6. Changes in plasma concentrations of atrial natriuretic factor (ANF) in rats receiving saline vehicle (□) and infusion of endothelin (▨) at 1 ng kg^{-1} min^{-1}. Statistical analysis was performed by an unpaired *t* test.

Groups 3 and 4: effect of endothelin on plasma concentrations of atrial natriuretic factor (ANF)

Figure 6 shows that infusion of endothelin at the dose of 1 ng kg^{-1} min^{-1} in group 4 did not affect plasma ANF concentrations (61 ± 16 pg ml^{-1}) compared with group 3 control experiments (66 ± 9 pg ml^{-1}).

TABLE 2. Effect of endothelin (1 ng kg^{-1} min^{-1}, Peptide Institute, Osaka, Japan) on mean arterial blood pressure and urinary excretion of water and electrolytes in group 5 rats

Variables	Control	Endothelin
MABP (mmHg)	110 ± 2	98 ± 4**
Hct (%)	$45 \pm 0{\cdot}6$	$45 \pm 0{\cdot}8$
P_{Na} (mmol l^{-1})	$142{\cdot}5 \pm 0{\cdot}6$	$142{\cdot}6 \pm 0{\cdot}7$
P_K (mmol l^{-1})	$3{\cdot}92 \pm 0{\cdot}2$	$3{\cdot}76 \pm 0{\cdot}3$
V (μl min^{-1})	$16{\cdot}3 \pm 1{\cdot}5$	$77{\cdot}3 \pm 4{\cdot}6$**
$U_{Na}V$ (μmol min^{-1})	$0{\cdot}83 \pm 0{\cdot}12$	$10{\cdot}66 \pm 1{\cdot}83$**
U_KV (μmol min^{-1})	$1{\cdot}23 \pm 0{\cdot}28$	$2{\cdot}17 \pm 0{\cdot}26$**

Values are means $\pm$ S.E.M. The data are analysed by Student's paired t test. *$P < 0{\cdot}05$; **$P < 0{\cdot}01$. Abbreviations are as in Table 1.

Group 5: effect of endothelin (Peptide Institute, Osaka, Japan) on MABP and urinary excretion of sodium and water

The data are summarized in Table 2 and compared with those obtained during infusion of the endothelin supplied by Auspep, Australia, at 1 ng kg^{-1} min^{-1}. Porcine endothelin from the Peptide Institute, Japan also produced similar hypotensive and natriuretic responses. MABP decreased by an average of 12 mmHg, whereas urine flow rate increased by 4-fold and urinary sodium excretion by 10-fold following infusion of endothelin. Potassium excretion also increased significantly. There were also no changes in arterial haematocrit or plasma concentrations of sodium and potassium.

DISCUSSION

Porcine endothelin was initially described as one of the most potent known vasoconstrictors (Yanagisawa *et al.* 1988). Subsequent studies suggested that its vasoconstrictor action might contribute to the pathogenesis of hypertension, vascular spasm and acute renal failure (Firth *et al.* 1988; Tomobe *et al.* 1988). The results reported here indicate an additional facet in the pattern of responses to endothelin and demonstrate that constant infusions of low concentrations decrease systemic arterial pressure and renal vascular resistance and cause marked diuresis and natriuresis. These data are consistent with a vasodilator action of endothelin reported by others using low doses *in vivo* or *in vitro* (Lippton *et al.* 1988; Wright & Fozard, 1988; Warner *et al.* 1989*a*: Minkes *et al.* 1989; Winquist *et al.* 1989*a*). Since endothelins synthesized in two different laboratories (Auspep, Australia, and Peptide Institute, Japan) induced similar responses, the hypotension, vasodilatation and natriuresis observed in the present study are unlikely to be due to contaminate in the synthetic peptide.

The systemic arterial pressure response to constant infusion of endothelin at low concentrations was characterized by an initial transient hypertension and subsequent hypotension. This sequence is the converse of many previous observations in which endothelin induced transient decreases in blood pressure followed by sustained hypertension (Wright & Fozard, 1988; Yanagisawa *et al.* 1988; Cocks *et al.* 1989; Minkes & Kadowitz, 1989; Winquist *et al.* 1989*a*). The simplest explanation for the difference relates to the dose and mode of administration. In the cited studies, much higher doses were administered by bolus injection (Yanagisawa *et al.* 1988; Wright & Fozard, 1989). The initial fall in blood pressure in those studies might be accounted for by decreased cardiac output and heart rate following a rapid increase in plasma endothelin concentrations (Goetz *et al.* 1988; Scoggins, Spence, Parks, McDonald, Wade & Coghlan, 1989). In contrast, in the present study low concentrations of endothelin (approximately 0·4–4 pmol min^{-1}) were infused over long periods thereby avoiding the development of transient high concentrations of the peptide. In our studies, the hypertensive response elicited immediately after infusion commenced could represent the direct vasoconstrictor action of the peptide, whereas the subsequent prolonged hypotension probably resulted from reduced vascular resistance as has been observed in mesenteric (Wright & Fozard, 1988; Minkes & Kadowitz, 1989; Warner *et al.* 1989*a*), femoral (Minkes *et al.* 1989) and renal vascular beds (Lippton *et al.* 1988; Wright & Fozard, 1988). We infer that the cardiovascular response to endothelin is dependent upon the dose and mode of administration, with the net effect on arterial blood pressure being the resultant of a direct vasoconstrictor action and an opposing vasodilatation which itself may be masked by high doses. In agreement with this we have observed in similar experiments a pressor response, renal vasoconstriction, and antinatriuresis during infusion of a dose (30 ng kg^{-1} min^{-1}) higher than those used in this study (D. Thomas, unpublished observations).

During the phase of stable systemic hypotension, calculated renal vascular resistance (RVR) fell and RPF rose markedly but GFR remained unchanged. The fall in FF could have been a consequence of pre- and postglomerular vasodilatation (Lippton *et al.* 1988; Wright & Fozard, 1988), but might also have involved a decrease in glomerular ultrafiltration coefficient (K_f). Endothelin receptors have been localized to renal glomeruli (Kohzuki *et al.* 1989; Orita *et al.* 1989) and although the intraglomerular target cells are not known with certainty, low doses of endothelin stimulate mitogenesis and activate the phosphoinositide cascade in cultured rat glomerular mesangial cells (Simonson *et al.* 1989). Endothelin might therefore have a direct action on glomerular mesangial cells, and in general phosphoinositide activation in these cells results in contraction consistent with a decrease in K_f.

Many previous studies have demonstrated that high concentrations of endothelin can reduce urine flow and sodium excretion due primarily to direct renal vasoconstriction (Firth *et al.* 1988; Goetz *et al.* 1988; King, Brenner & Anderson, 1989; Lopez-Farre *et al.* 1989; Miller *et al.* 1989). In the present study the renal effects of lower doses of endothelin were characterized by profound diuresis and natriuresis despite systemic hypotension and consequent decreased renal perfusion pressure. Urine flow rate and fractional water excretion increased approximately 5-fold, whereas absolute and fractional sodium excretion increased by more than 10-fold.

The effects were not dose-dependent and it may be inferred that the lower dose (1 ng kg^{-1} min^{-1}) elicited a maximal response. The marked diuresis and natriuresis induced by endothelin in the present study cannot be accounted for by increased filtered load, but must therefore relate to suppression of renal tubular reabsorption.

The lithium clearance data provide evidence for a proximal site of action of endothelin. In the face of unchanged filtered load, solute and fluid delivery from the end of the proximal nephron doubled, and fractional proximal sodium reabsorption decreased by 10% with the low dose and by 20% with the higher dose. The mechanisms responsible for these proximal effects are not clear but might include changes in peritubular physical forces or direct and indirect actions of endothelin on the tubular cells. The decrease in filtration fraction would be expected to reduce peritubular oncotic pressure with consequent attenuation of fluid uptake. A direct action of endothelin on proximal transport is also a strong possibility since high-affinity binding sites have been demonstrated in the proximal convoluted tubules (Kohzuki *et al.* 1989).

Despite a doubling of end-proximal delivery of sodium and fluid into the distal nephron segments, fractional distal reabsorption of sodium was attenuated by 5–7% ($P < 0.01$) during endothelin infusions. The site of this inhibition is not clear but neither the diluting segments nor the ADH-sensitive distal tubules and collecting ducts appear to be implicated. Thus, although osmolar clearance doubled during infusion of endothelin, solute-free water clearance remained unaltered. Furthermore, endothelin does not alter plasma concentrations of vasopressin (Goetz *et al.* 1988). An alternative mechanism for the distal action of endothelin is based on the distribution of binding sites in the inner medulla and overlying the vasa recta bundles in the outer medulla (Kohzuki *et al.* 1989). Endothelin may bind with these putative receptors and cause redistribution of intrarenal blood flow and GFR, contributing to the observed reduction of fractional distal reabsorption. Moreover, there is evidence that endothelin inhibits Na^+–K^+-ATPase in the inner medullary collecting duct (Zeidel *et al.* 1989). These combined effects could account for the endothelin-induced supression of fractional distal reabsorption.

The mechanisms by which low doses of endothelin elicit systemic and renal vasodilatation and natriuresis may involve interactions with multiple circulating or local hormones and/or humoral mediators. The renal responses, particularly the marked natriuresis and suppression of fractional proximal reabsorption, are similar in several respects to those seen with intravenous infusions of low doses of ANF (Harris *et al.* 1989) or angiotensin-converting enzyme inhibitors (Zhuo, Harris & Skinner, 1989). The observations that endothelin stimulates ANF release from cultured rat myocytes (Fukuda, Hirata, Yoshimi, Kojima, Kobayashi, Yanagisawa & Masaki, 1988) and isolated right atria (Winquist, Scott & Vlasuk, 1989*b*) suggested that increased plasma ANF concentration might have been responsible for the renal effects of endothelin in the present study. However, infusion of endothelin did not alter plasma ANF concentration compared with the time-control experiments and additional mediators must be considered.

Endothelin has been shown to inhibit renin secretion from isolated rat glomeruli (Rakugi, Nakamaru, Saito, Higaki & Ogihara, 1988) and a reduction in renin release would be expected to decrease circulating and intrarenal angiotensin II con-

centrations thereby contributing to the decreased proximal tubular reabsorption. But the extent of the natriuresis would imply a total suppression of the renin–angiotensin system, similar to that produced acutely by enalaprilat (Harris, Zhuo & Skinner, 1987) which would seem most unlikely but which will require further elucidation. Nevertheless, the overlap of receptor binding sites for endothelin, ANF and angiotensin II within the kidneys including glomeruli, proximal tubules, vasa recta bundles and inner medulla provides a basis for interactions between these peptides in normal renal function which must be addressed in future work (Chai, Sexton, Allen, Figdor & Mendelsohn, 1986; Mendelsohn, Dunbar, Allen, Chou, Millan, Aguilera & Catt, 1986; Kohzuki *et al.* 1989; Neuser, Steinke, Theiss & Stasch, 1989). Other possibilities arise from reports that endothelin induces release of vasodilators such as endothelium-derived relaxing factor (EDRF) and prostacyclin (de Nucci, Thomas, D'Orleans-Juste, Antunes, Walder, Warner & Vane, 1988; Warner, Mitchell, de Nucci & Vane, 1989*b*) which have hypotensive, vasodilator and natriuretic actions.

We conclude that as the intravenous dose of endothelin is reduced towards its predicted physiological level in the anaesthetized rat, its actions become predominately depressor, vasodilator and natriuretic. These effects may, at least in part, be mediated indirectly by release of, or by interaction with, other vasoactive substances.

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