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Endothelin-1 in the rabbit: interactions with cyclo-oxygenase and NO-synthase products

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1 Endothelin-1 infusion (5–40 pmol kg^{-1} min^{-1}) in the normal anaesthetized rabbit, produced a dose-dependent increase in mean arterial blood pressure (MAP) and reduced renal blood flow (RBF) and glomerular filtration rate (GFR), when compared with an equivalent infusion of physiological saline.

2 Endothelin, 20 pmol kg^{-1} min^{-1}, was also assessed in animals pretreated with either indomethacin (2 mg kg^{-1}), methylene blue (1.6 mg kg^{-1} h^{-1}) or N^G-monomethyl L-arginine (L-NMMA, 10 mg kg^{-1} h^{-1}).

3 The effect of endothelin on MAP and RBF was enhanced ($P = 0.05$ and < 0.01 respectively) by the cyclo-oxygenase inhibitor, indomethacin, without any significant change in the effect on GFR.

4 Methylene blue and L-NMMA, inhibitors of endothelium-derived relaxant factor (EDRF), enhanced the effect of endothelin on each of the parameters measured ($P < 0.01$).

5 Our results are consistent with endothelin having a predominant effect on pre-glomerular vascular resistance to reduce GFR. Endothelin appears to stimulate the release of vasodilator prostanoids and EDRF which oppose its effects. Thus endothelin may have an important role in the complex control of GFR in the rabbit.

Keywords: Endothelin; endothelium-derived relaxing factor; glomerular filtration rate; NO synthase; cyclo-oxygenase

Introduction

Endothelin-1 is a 21 amino acid peptide with potent vasoconstrictor properties (Yanagisawa *et al.*, 1988). It is produced by endothelial cells in culture and is coded in the genome of several species. Endothelium-dependent constricting factors are released in response to stimuli such as hypoxia and increased transmural pressure (Rubanyi & Vanhoutte, 1985) and endothelin is thought to be one such factor. Endothelin has a prolonged effect *in vivo* but a short half-life in the circulation (Spokes *et al.*, 1989). This is consistent with avid uptake at specific binding sites, which have been demonstrated widely in the vasculature and in the parenchyma of several organs including kidney, brain and heart (Koseki *et al.*, 1989).

Endothelin increases renal vascular resistance and decreases renal function in both rats (Badr *et al.*, 1989; López-Farré *et al.*, 1989) and dogs (Goetz *et al.*, 1988; Miller *et al.*, 1989). Kon and her colleagues (1989) infused endothelin-1 into the first order branch of the left main renal artery in Munich-Wistar rats and found a 35% reduction in single nephron glomerular filtration rate (GFR) compared with unexposed glomeruli in the same kidney. Furthermore, in models of ischaemic acute renal failure (Kon *et al.*, 1989) and acute cyclosporin nephrotoxicity (Kon *et al.*, 1990) in the same model, the infusion of a polyclonal anti-endothelin antibody protected against the characteristic renal vasoconstriction in each case. This supports the idea that endothelin might have an important physiological and pathophysiological role in the kidney (Firth *et al.*, 1988; Cairns *et al.*, 1988).

The control of glomerular blood flow is critical in the maintenance of homeostasis and the rabbit kidney exhibits autoregulation of its flow (Forster & Maes, 1947). There is a complex interaction between neural and humoral, constrictor and dilator and local and systemic mechanisms contributing to this control. The two best documented regulatory mechanisms involved are the renin-angiotensin system and the renal eicosanoids; the principal renal prostaglandins are prostaglandin E_2 (PGE_2) and PGI_2 (Terragno *et al.*, 1978), which are both vasodilator in the kidney. Since the isolated perfused kidney can still autoregulate (Kastner *et al.*, 1984), local factors are likely to be important, in particular, the importance of the endothelium as a source of vasoactive agents is increasingly recognized.

We have therefore studied the systemic and renal effects of endothelin in the rabbit and assessed its role in the regulation of renal blood flow by inhibiting some of these other systems: cyclo-oxygenase with indomethacin and the action and generation of endothelium-derived relaxing factor (EDRF: nitric oxide, Ignarro *et al.*, 1987), with methylene blue (MB) an inhibitor of soluble guanylate cyclase (Ignarro *et al.*, 1986) and N^G monomethyl-L-arginine (L-NMMA) a competitive inhibitor of the enzyme NO synthase (Palmer & Moncada, 1989).

Methods

Surgical preparation

Normal adult New Zealand White rabbits (6–10 in each group) of either sex and weighing 2.5–3.5 kg (Rosemead, U.K.) were fluid loaded with physiological saline (50 ml) and given 2 mg frusemide via the ear vein, to induce a temporary diuresis prior to cannulation of the ureter. Anaesthesia was induced with pentobarbitone 15 mg kg^{-1} (Sagatal, May & Baker, Dagenham, U.K.) and althesin 0.2 ml kg^{-1} (containing 9 mg alfaxalone and 3 mg alphadolone ml^{-1}) (Saffan, Glaxovet, U.K.) as intravenous boluses, and maintained with a continuous infusion of althesin 1.2–1.5 ml kg^{-1} h^{-1} into

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the ear vein.

After formation of a tracheostomy, the animals were ventilated with room air supplemented with 100% oxygen. The left carotid artery was cannulated for blood sampling and for continuous measurement of blood pressure via a Statham blood pressure transducer on a Watanabe (Type WA281) pen chart recorder. A second catheter was introduced by the Seldinger technique, over a guide wire, into the right femoral artery and thence into the aorta. The catheter tip was positioned approximately one centimeter above the level of the renal arteries by palpation and was flushed with heparinised saline for the subsequent infusion of endothelin.

At laparotomy both ureters were cannulated so that timed urine collections could be made and an electromagnetic flow probe (1.5 mm, Gould) was placed around the left renal artery and connected to a flowmeter (Gould Inc., Oxnard, Ca. model SP2202) for measurement of renal blood flow. The midline wound was then closed with clips. Animals were hydrated throughout with 0.9% saline, 20 ml kg^{-1} h^{-1}, and 0.5 MBq of [^{51}Cr]-EDTA was administered by the carotid cannula as a bolus, followed by a further 0.5 MBq in isotonic saline as a continuous infusion (0.3 ml min^{-1}), via the ear vein.

In addition in six animals, indomethacin was given as an intravenous bolus (2 mg kg^{-1}) followed by an infusion at 1 mg kg^{-1} h^{-1} in normal saline, to block the cyclo-oxygenase enzyme (Flower, 1974).

Methylene blue (MB), 1.6 mg kg^{-1} h^{-1} and L-NMMA, 10 mg kg^{-1} h^{-1}, were infused for 140 and 60 min respectively from the end of the recovery period in a further 5 animals each. These doses had been shown in our preliminary experiments to inhibit the effect of acetylcholine 10 nmol kg^{-1} min^{-1} in the normal rabbit.

Experimental procedure

After completion of the surgical preparation and infusions of [^{51}Cr]-EDTA and drugs, the animals were allowed to recover from the operation for a period of 30–40 min. Two consecutive baseline 10 min collections of urine were then made with recordings of arterial pressure (MAP) renal blood flow (RBF) and plasma sampling at the mid-point of the collection. Glomerular filtration rate (GFR) was subsequently calculated as the clearance of [^{51}Cr]-EDTA during each collection period. In the animals treated with methylene blue or L-NMMA, repeateed baseline measurements were made after 10 min of administration. Endothelin-1 (Peptide Institute, Osaka, Japan), 250 nM in normal saline, was then infused into the aortic cannula to allow adequate mixing of the drug in the blood before reaching the renal circulation whilst minimizing the systemic effect. The infusion continued for a total of 40 min during which three sets of measurements were made at 5, 10 and 30 min respectively.

After completing the infusion a 70 min recovery period was allowed with three similar observation periods at 55, 80 and 110 min from the start of the infusion. Endothelin was infused at 5, 20 and 40 pmol kg^{-1} min^{-1} in preliminary studies to assess the dose response ($n = 5–6$) and thereafter, 20 pmol kg^{-1} min^{-1} was infused in each experiment.

Results are expressed, at each time point, as the percentage change from the mean of the two baseline measurements (absolute baseline values are shown in Table 1). The GFR results reported are from the right kidney. The rabbit renal artery is sensitive to manipulation and placement of the flow probe on the left resulted in a reduction in renal function on that side which recovered to a variable degree in each case. In 27 of 122 different experiments employing this preparation renal function was preserved on the left (mean GFR 69.2% of that on the right, range 10.7–268%). In these cases both kidneys responded similarly to pharmacological manipulation, and we have therefore considered it valid to compare measurements of function on the right with recordings of blood flow from the left kidney.

Statistical analyses were by multifactor analysis of variance (ANOVA).

Results

Endothelin alone

Endothelin infusion (20 pmol kg^{-1} min^{-1}) produced an increase in MAP from baseline 49.3 ± 5 mmHg to 56.8 ± 5 mmHg (mean ± s.e.mean) at the end of the infusion period. This was accompanied by a reduction in GFR (4.2 ± 0.5 to 2.1 ± 0.5 ml min^{-1}) and RBF (12.9 ± 2.3 to 8 ± 2 ml min^{-1}) (Figure 1). The changes were dose related, in the range 5–40 pmol kg^{-1} min^{-1}, developed immediately and progressed throughout the course of the infusion period to a maximum at 30 min in most cases. There was no initial fall in MAP. After the infusion, mean arterial pressure returned to baseline values by 55 min at the lower dose levels of endothelin but did not recover after 40 pmol kg^{-1} min^{-1}. RBF was not affected by the lowest dose of endothelin

Table 1 Absolute data at baseline before infusion of control saline or endothelin at various doses with or without other named agents

	MAP (mmHg)	*RBF* (ml min^{-1})	*GFR* (ml min^{-1})	*PP* (mmHg)
Control	53.1 (3.1)	17.7 (2.6)	3.3 (0.6)	33.7 (3.0)
Endothelin 20 pmol kg^{-1} min^{-1}	49.3 (5.1)	12.8 (2.3)	4.2 (0.5)	29.9 (2.4)
Endothelin 40 pmol kg^{-1} min^{-1}	44.0 (5.0)	20.0 (5.3)	3.1 (1.0)	26.3 (5.9)
Endothelin 5 pmol kg^{-1} min^{-1}	70.2 (4.8)	16.6 (2.4)	5.1 (0.5)	33.6 (2.1)
Endothelin 20 + indomethacin	69.8 (2.7)	25.9 (3.7)	5.8 (1.0)	26.6 (2.1)
Endothelin 20 + Meth. blue	69.9 (1.7)	15.1 (2.4)	4.5 (1.0)	29.7 (1.8)
Endothelin 5 + Meth. blue	74.8 (5.8)	14.4 (4.0)	1.7 (0.3)	29.9 (2.3)
Endothelin 1 + Meth. blue	66.5 (11.2)	16.1 (3.3)	3.0 (0.8)	28.3 (2.9)
Endothelin 20 + L-NMMA	74.5 (6.9)	24.7 (3.0)	4.8 (0.9)	25.6 (3.0)

Values are mean ± s.e.mean. MAP = mean arterial pressure; RBF = renal blood flow; GFR = glomerular filtration rate; PP = pulse pressure. L-NMMA = N^G-monomethyl L-arginine; Meth blue = methylene blue.

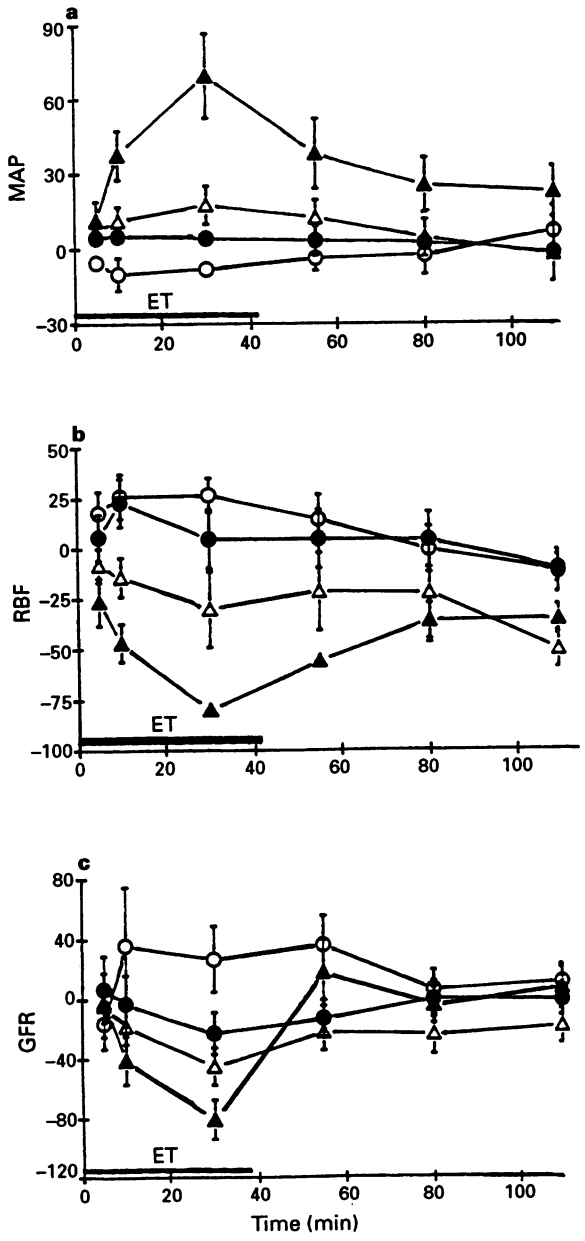


Figure 1 Dose-response curve to a 40 min endothelin-1 infusion. (a) Percentage change in mean arterial pressure (MAP); (b) percentage change in renal blood flow (RBF); (c) percentage change in glomerular filtration rate (GFR): (○) saline control; (●) endothelin 5 pmol kg^{-1} min^{-1}; (△) endothelin 20 pmol kg^{-1} min^{-1}; (▲) endothelin 40 pmol kg^{-1} min^{-1}. Horizontal line indicates endothelin infusion period.

(5 pmol kg^{-1}) but at 40 pmol kg^{-1} min^{-1} the marked reduction (80%) had not returned to the baseline value by the end of the observation period. In contrast, there was a fall in GFR after only 5 pmol kg^{-1} endothelin ($P = 0.06$ v control) and the profound reduction in GFR (81 ± 13.2%) induced by endothelin 40 pmol kg^{-1} min^{-1} returned to the pre-infusion level 15 min after the infusion had been discontinued. A progressive fall in pulse pressure also occurred throughout the infusion and did not recover (data not shown).

Endothelin with indomethacin

Administration of indomethacin alone increases basal blood pressure and GFR (24.8 and 15.4% respectively, $n = 3$); results following infusion of endothelin were therefore expressed as percentages from this new baseline. Indomethacin enhanced the effect of endothelin infusion (20 pmol kg^{-1}) on RBF (25.9 ± 3.7 ml min^{-1} falling to 8 ± 1.4 ml min^{-1}) (that is − 66.5% of baseline compared with − 30.3%, $P = 0.02$) and MAP (69.8 ± 2.7 to 88.9 ± 3.5 mmHg or 27.4% against 17.8% for endothelin alone, $P = 0.35$), neither MAP nor RBF had returned to pre-infusion values by the end of the experiment. Despite these changes, the fall in GFR after administration of indomethacin (5.6 ± 0.9 to 2.6 ± 0.6 ml min^{-1}) was not different from that produced by endothelin alone (Figure 2).

Endothelin with methylene blue and N^G-monomethyl-L-arginine

MB potentiated the effect of endothelin on MAP (+ 57% v 17.8%, $P<0.01$), RBF (− 75% v − 30.3%, $P<0.001$) and GFR (− 91.4% v − 45%, $P<0.001$) (Figure 3). A dose-response curve to endothelin (1–20 pmol kg^{-1} min^{-1}) in the presence of MB showed a shift to the left (Figure 4) indicating that endothelin is more potent in the absence of soluble guanylate cyclase activity. Similarly, in the presence of L-NMMA 10 mg kg^{-1} h^{-1}, all three parameters showed an exaggerated response to endothelin 20 pmol kg^{-1} min^{-1} (MAP + 39%, $P<0.01$; RBF − 74%, $P<0.02$; GFR − 87%, $P<0.02$, Figure 3). L-NMMA also caused a basal increase in systemic blood pressure of 10%, but without a statistically significant change in RBF (data not shown). There was no difference between the effects of MB and L-NMMA on the response to endothelin 20 pmol kg^{-1} min^{-1} ($P<0.6$), thus this enhancement of the effect of endothelin is likely to be due to inhibition of EDRF.

Control

Infusion of physiological saline at the same rate as endothelin served as the control experiment (Figure 1), and produced a 10% fall in MAP and a rise in RBF (20%) and GFR (35%).

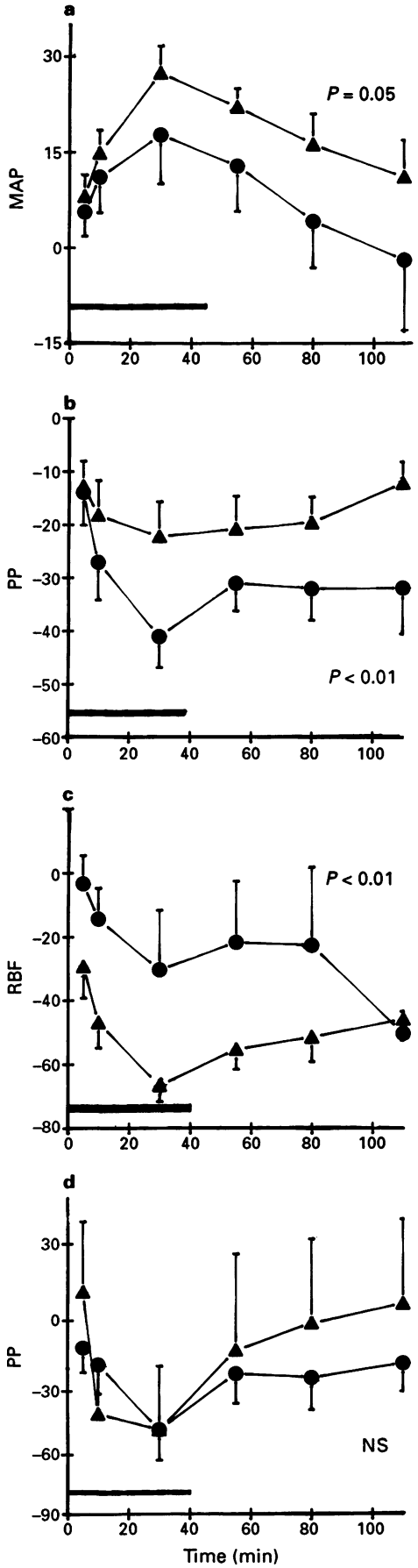
These changes may represent the effect of a local infusion of fluid into the aorta, but more likely represent the instability of the baseline values, since they did not progress during the infusion. The changes seen in response to endothelin are opposite to those in the control animals, hence any error introduced by the early response to saline will tend to minimize the observed effect of endothelin, enhancing the validity of our conclusions.

Discussion

Our results demonstrate that endothelin is a very potent constrictor of the systemic and renal vasculature. The constrictor effect is markedly potentiated by inhibition of either the cyclo-oxygenase enzyme system or the action or generation of EDRF, indicating that vasodilator prostaglandins and EDRF are released *in vivo* to offset the effect of endothelin alone. The reduction of GFR induced by endothelin is also potentiated by inhibitors of EDRF but not by indomethacin suggesting that the interaction of endothelin and cyclo-oxygenase products in the control of GFR is more complex.

The reduction in renal perfusion and glomerular filtration rate observed *in vivo*, confirms our earlier finding in the isolated perfused kidney of the rabbit (Cairns *et al.*, 1989) and is consistent with other studies of endothelin infusion *in vivo*, in both rat (Badr *et al.*, 1989) and dog (Goetz *et al.*, 1988; Miller *et al.*, 1989) and with the effect of endothelin infused at low dose (0.16 pmol min^{-1}) directly into a segmental renal artery in rats (Kon *et al.*, 1989). The dose-related reduction in renal blood flow and GFR, despite a significant increase in mean arterial blood pressure, suggests that

endothelin is acting predominantly on pre-glomerular resistance vessels to reduce the net intraglomerular hydraulic pressure difference (Δ-P), and glomerular plasma flow. Preferential afferent arteriolar constriction by endothelin has been shown by direct observation in the split hydronephrotic rat kidney model (Loutzenhiser *et al.*, 1990). Micropuncture studies in Munich-Wistar rats (Badr *et al.*, 1989) have confirmed that endothelin infusion (10 pmol min^{-1}) increases the resistance of both afferent and efferent arterioles, lowering both the single nephron GFR and the calculated ultrafiltration co-efficient (K_f). In support of the latter, endothelin has been shown to induce contraction of mesan-



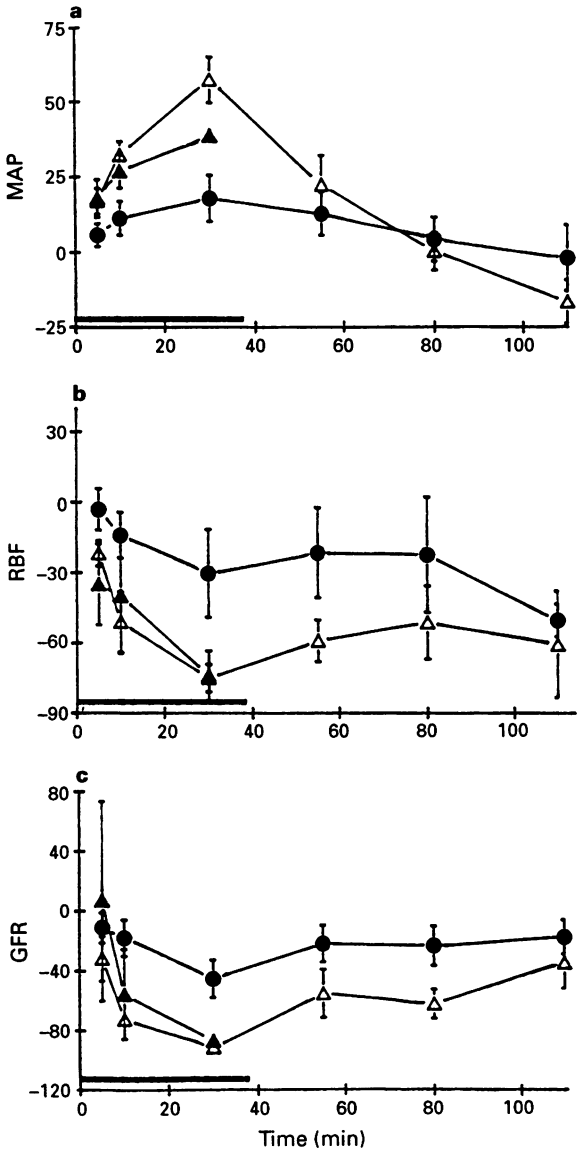


Figure 3 Percentage change in (a) mean arterial blood pressure (MAP); (b) renal blood flow (RBF) and (c) glomerular filtration rate (GFR) following infusion of endothelin 20 pmol kg^{-1} min^{-1} alone (●); endothelin 20 pmol kg^{-1} min^{-1} with methylene blue 1.6 mg kg^{-1} h^{-1} (Δ); endothelin 20 pmol kg^{-1} min^{-1} with N^G-monomethyl-L-arginine 10 mg kg^{-1} (▲). Horizontal line indicates endothelin infusion.

Figure 2 Percentage change in (a) mean arterial blood pressure (MAP), (b) pulse pressure (PP), (c) renal blood flow (RBF) (d) glomerular filtration rate (GFR) following infusion of endothelin 20 pmol kg^{-1} min^{-1} alone (●); endothelin 20 pmol kg^{-1} min^{-1} with indomethacin 2 mg kg^{-1} (▲). Horizontal line indicates endothelin infusion period.

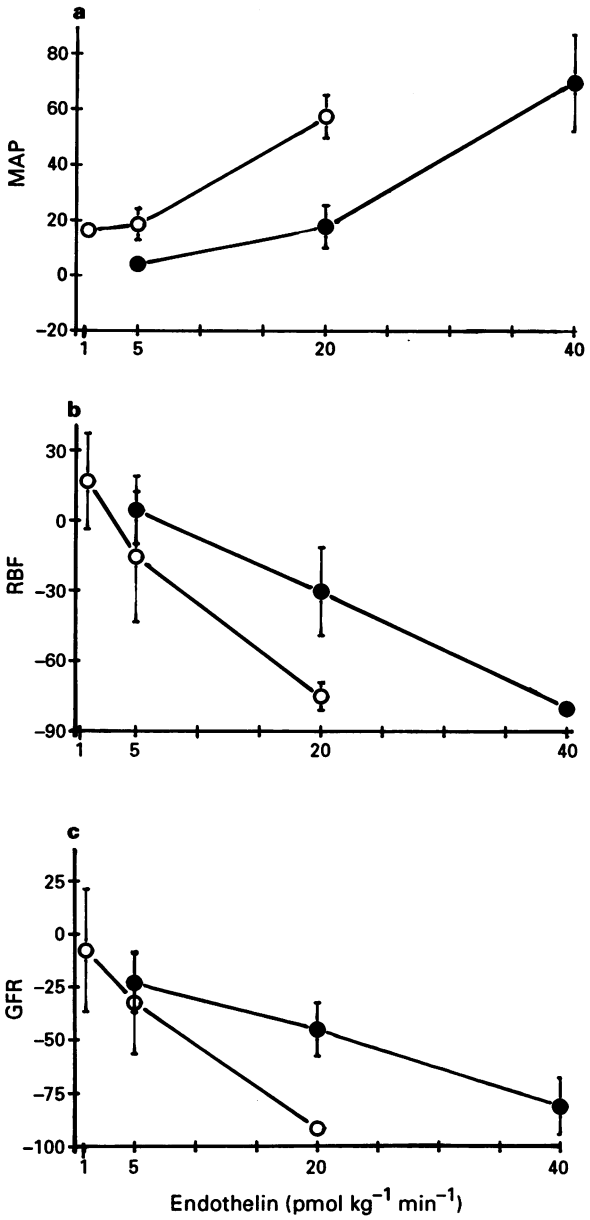


Figure 4 Maximal change in (a) mean arterial pressure (MAP), (b) renal blood flow (RBF) and (c) glomerular filtration rate (GFR), at variable doses of endothelin, with or without methylene blue (1.6 mg kg^{-1} h^{-1}): (●) endothelin alone; (○) endothelin with methylene blue 1.6 mg kg^{-1} h^{-1}.

gial cells in culture (Badr *et al.*, 1989; Simonson *et al.*, 1989), Kon however, found no change in K_f (Kon *et al.*, 1989). Other studies in anaesthetized rabbits also showed an increase in both pre and post glomerular vascular resistances, with a delayed fall in GFR (Denton & Anderson, 1990). The overall effect of endothelin on GFR may therefore be complex, involving changes in both vascular resistance and mesangial cell tone.

The striking reduction in pulse pressure seen during endothelin infusion implies that its hypertensive effect may be mediated by an effect on the peripheral vasculature rather than on cardiac function. However an additional inotropic effect, either positive or negative, cannot be excluded.

Endothelin stimulates the release of prostaglandins from guinea-pig and rat isolated lungs (de Nucci *et al.*, 1988), rat mesenteric arteries (Warner *et al.*, 1989) and from rabbit isolated perfused kidney and spleen (Rae *et al.*, 1989), and it is known to activate phospholipase A_2 in isolated vascular smooth muscle cells in culture (Resink *et al.*, 1989). Our results show that indomethacin enhances the constrictor effects of endothelin infusion (20 pmol kg^{-1} min^{-1}) both systemically and in the kidney, suggesting that indomethacin has inhibited the production of predominantly vasodilator eicosanoids. Some of this effect might also be explained by a switch of arachidonic acid from the cyclo-oxygenase to the 5-lipoxygenase pathway and the production of constrictor mediators. In contrast to RBF, GFR is preserved in the presence of indomethacin indicating that the control of GFR is more complex than just the change in renal vascular resistance and intraglomerular hydrostatic pressure, and may involve other parameters such as mesangial tone where the balance between eicosanoids and other mediators including endothelin is different. Whether phopholipase A_2 is directly activated by endothelin or whether this is a secondary response to the haemodynamic changes induced by the infusion *in vivo* is unknown.

Methylene blue and L-NMMA are inhibitors of the action and formation, respectively, of EDRF. EDRF, which is now characterized as nitric oxide, is a short lived vasodilator released from the vascular endothelium in response to shear stress as well as pharmacological stimuli such as acetylcholine and substance P (Vanhoutte, 1988). The role of EDRF in the control of vascular tone *in vivo* is not yet established although a dose-related increase in basal blood pressure has been reported in rabbits following infusion of L-NMMA (Rees *et al.*, 1989), suggesting a basal dilator tone induced by EDRF. Our results confirm the haemodynamic effect on basal systemic tone (MAP increased by 10% following L-NMMA) but without change in renal blood flow. This suggests that EDRF may have a role in the control of resting systemic blood pressure, but in this model its role in the renal circulation is more impressive in pathological states e.g. in reversing the effect of a potent vasoconstrictor substance such as endothelin.

We did not observe a preliminary vasodilator effect following endothelin infusion in the rabbit. In rats and dogs, such an effect has been attributed to release of dilator prostaglandins and EDRF. Our results suggest that both of these systems are activated throughout the course of the endothelin effect, but that the constrictor effect is dominant.

In summary, we have shown that endothelin is a potent vasoconstrictor in the rabbit, and that it reduces glomerular filtration rate, probably by preferential constriction of the afferent renal arteriole. The response to endothelin is modulated *in vivo* by the production of vasodilator prostaglandins and EDRF and to a lesser extent by activation of the renin-angiotensin system (Rogerson *et al.*, 1989). The cellular or sub-cellular level of this interaction remains to be determined. The control of renal vascular resistance and glomerular filtration rate involves a complex network of many factors. Our results suggest that endothelin-1 may be one of these.

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