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Support of renal blood flow after ischaemic-reperfusion injury by endogenous formation of nitric oxide and of cyclo-oxygenase vasodilator metabolites

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1 Ischaemia-reperfusion injury in the kidney is associated with a loss of autoregulation, an increase in renal vascular resistance (RVR), a decrease of renal blood flow (RBF) and ultimately acute renal failure. The aim of this study was to investigate the role of the release of endogenous nitric oxide (NO) in the recovery of RBF after ischaemic injury of the renal vascular bed.

2 Anaesthetized rats (thiopentone sodium; 120 mg kg^{-1}, i.p.) were submitted to acute renal ischaemia followed by 2 or 6 h of reperfusion (I/R). Reperfusion was associated with a significant reduction in RBF, an increase in RVR, and an impairment of the vasodilator effect of acetylcholine (ACh).

3 N^G-nitro-L-arginine methyl ester (L-NAME, 30 $\mu g\ kg^{-1}\ min^{-1}$, i.v., $n = 5$) significantly prevented the recovery of RBF after I/R injury. Similarly, inhibition of prostanoid formation with indomethacin (5 mg kg^{-1}, i.v., $n = 4$) significantly enhanced the rise in RVR associated with I/R injury.

4 Infusion of L-arginine (L-Arg; 1 or 3 mg $kg^{-1}\ min^{-1}$, i.v., $n = 5$ and 4, respectively) or D-Arg (1 mg $kg^{-1}\ min^{-1}$, i.v., $n = 6$), starting 30 min after occlusion, did not improve the recovery of RBF. Furthermore, infusion of L-Arg (20 mg $kg^{-1}\ min^{-1}$ for 15 min; $n = 4$) had no effect on the I/R-induced impairment of the vasodilator responses to ACh.

5 To elucidate the relative importance of the constitutive and inducible NO synthase isoforms for the formation of NO after I/R, calcium-dependent (constitutive) and calcium-independent (inducible) NO synthase activities were measured in kidney homogenates obtained from ischaemic or non-ischaemic kidneys. A calcium-independent NO synthase activity was not detectable in kidney homogenates obtained from either sham-operated control rats or from animals subjected to I/R. Moreover, dexamethasone (3 mg kg^{-1}, i.v., 60 min prior to I/R, $n = 6$), an inhibitor of the induction of NO synthase, had no effect on either RBF or RVR in rats subjected to I/R. In contrast to I/R, lipopolysaccaride (LPS, endotoxin; 5 mg kg^{-1}, i.p., $n = 3$) caused a significant induction of a calcium-independent NO synthase activity in the kidney.

6 These results confirm the importance of the release of vasodilator cyclo-oxygenase metabolites in the compromised renal circulation and indicate that the formation of NO derived from the constitutive, but not the inducible NO synthase, is also important for the maintenance of RBF after I/R injury of the renal vascular bed.

Keywords: Nitric oxide; renal ischaemia; endothelium; L-arginine; N^G-nitro-L-arginine methyl ester

Introduction

The formation of endothelium-derived nitric oxide (NO) from the guanidino nitrogen group of L-arginine (L-Arg) is important for the regulation of blood pressure and microcirculatory blood flow in a variety of species including man (see Moncada *et al.*, 1991; Thiemermann, 1991; Vane & Botting, 1992). There are several distinct enzymes responsible for the formation of NO (Förstermann *et al.*, 1991) including a constitutive, calcium-calmodulin-dependent NO synthase in endothelial (Pollock *et al.*, 1991) and neuronal cells (Bredt & Snyder, 1990). A calcium-independent NO synthase can be induced by cytokines in various cells including renal mesangial cells (Stuehr & Griffith, 1992). In macrophages, the biosynthetic pathway of NO synthesis from L-arginine includes an initial N-oxidation step resulting in the formation of N^G-hydroxy-L-arginine (Stuehr *et al.*, 1991). In addition, hydroxy-L-arginine is a substrate for NO synthase in cultured endothelial cells (Zembowitz *et al.*, 1991) and neuronal NO synthase from rat brain (Mitchell *et al.*, 1992). NO derived from the constitutive endothelial cell NO synthase regulates blood pressure and microcirculatory blood flow, while NO derived from the cytokine-inducible NO synthase is responsible for macrophage cytotoxicity (Marletta *et al.*, 1988; Moncada *et al.*, 1991), and contributes to the circulatory failure associated with endotoxic shock (Julou-Schaeffer *et al.*, 1990; Salter *et al.*, 1991; Szabo *et al.*, 1993).

A continuous basal release of NO within the renal vasculature is of major importance for the regulation of renal blood flow and function under physiological conditions in the anaesthetized (Tolins *et al.*, 1990; Walder *et al.*, 1991; Welch *et al.*, 1991; Zatz & De Nucci, 1991) or conscious (Baylis *et al.*, 1990) rat, as well as in the anaesthetized or conscious dog (Kiyomoto *et al.*, 1992). In addition, the substantial fall in renal blood flow (RBF) induced by inhibitors of NO formation, such as N^G-monomethyl-L-arginine (L-NMMA) or N^G-nitro-L-arginine methyl ester (L-NAME), in the anaesthetized rat can be prevented by both L-arginine and L-hydroxy-arginine, supporting the hypothesis that L-hydroxy-arginine is an intermediate in the formation of NO from L-arginine in the renal vasculature *in vivo* (Walder *et al.*, 1992). Moreover, perfusion of isolated kidneys of the rat with an L-arginine-free buffer solution is associated with a marked reduction in renal perfusion, suggesting that the supply of L-Arg may become rate-limiting, and that reduced supply of L-Arg may affect the regulation of RBF (Radermacher *et al.*, 1991). The vasodilator effect of acetylcholine in the isolated perfused kidney of the rat is dependent on the formation of NO (Radermacher *et al.*, 1990; Bhardwaj & Moore, 1989) resulting in an enhanced formation of guanosine 3':5'-cyclic monophosphate (cyclic GMP) by either vascular smooth muscle or renal mesangial cells or both (Burton *et al.*, 1990; Chevalier *et al.*, 1992).

Thus, under physiological conditions the basal release of

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NO is important for the regulation of RBF and function but less is known about the role of NO after ischaemia- and reperfusion-induced (IR) injury. Inhibition of NO formation aggravates the acute renal failure associated with administration of radiocontrast dyes in the anaesthetized rat (Brezis *et al.*, 1991). In addition, I/R injury releases cytokines (Holtz & Goetz, 1992; Ascer *et al.*, 1992) which induce NO synthase in other cells. Here we investigate the role of NO in the maintenance of RBF after I/R injury of the kidney in the anaesthetized rat. The relative contributions of constitutive and cytokine-inducible NO synthase isoforms for the maintenance of RBF after I/R injury were evaluated by inhibition of both NO synthase isoforms with L-NAME (Moore *et al.*, 1990; Hecker *et al.*, 1990; Thiemermann *et al.*, 1990); inhibition of NO synthase induction by dexamethasone (Radomski *et al.*, 1990; Knowles *et al.*, 1990); and by measurement of calcium-dependent (constitutive) and calcium-independent (inducible) NO synthase activities in tissue homogenates from non-ischaemic (control) and I/R kidneys (Bredt & Snyder, 1990; Mitchell *et al.*, 1991; Salter *et al.*, 1991). In addition, the contribution of cyclo-oxygenase metabolites to the recovery and maintenance of RBF after I/R injury has been assessed.

Methods

Surgical procedure

Male Wistar rats (350 to 450 g) were anaesthetized with thiopentone sodium (120 mg kg^{-1}, i.p.). The trachea was cannulated to facilitate respiration and body temperature was maintained at 37°C by means of a rectal probe connected to a homeothermic blanket (Bioscience, Sheerness, Kent). The right carotid artery was cannulated and connected to a transamerica type 4-422-001 pressure transducer for the measurement of mean arterial blood pressure (MAP) on a Grass 7D polygraph (Grass Instruments, Quincy, Mass., U.S.A.). The left jugular vein was cannulated for the administration of drugs and the right femoral vein for the administration of saline (1.5 ml h^{-1}) to compensate for any fluid loss.

The left kidney was exposed via a mild-line laparotomy and the renal artery isolated. An ultrasonic flow probe (1RB, internal diameter = 1 mm), embedded in a silicone cuff to provide optimum alignment, was placed around the left renal artery for measurement of total renal blood flow (RBF) using a Transonic T206 Small Animal Flowmeter (Transonic Systems Inc., New York, U.S.A.). A small amount of acoustical couplant (100 mg Nalco 1181, mixed with 10 ml distilled water; Nalco Chemical Co., IL., U.S.A.) was deposited in the probe's acoustic window adjacent to the artery, to replace all air. This flowmeter system uses an ultrasonic transit time principle, which provides a continuous real-time measure of volume flow, expressed in ml min^{-1}. Ultrasound Doppler flowmetry was chosen to measure RBF, because other techniques (hippuran clearance) result in artefactual and, hence, invalid estimations of RBF in conditions of ischaemia-induced acute renal failure (Stein *et al.*, 1978). Renal vascular resistance (RVR) was calculated by dividing MAP by RBF. After surgery, all animals were allowed to stabilize for 30 to 45 min before occlusion of the renal artery. A vascular clamp was positioned around the left renal artery between its origin and the ultrasonic flow probe. The clamp was then tightened to stop RBF. After 60 min of occlusion, the clamp was reopened to allow reperfusion for 2 to 6 h. In addition, some animals were submitted to the same surgical procedure except occlusion of the renal artery (sham-operated animals, SOP, $n = 3$). Haemodynamic parameters were continuously monitored throughout the experimental period. At the end of the experiment, both kidneys were removed, weighed and frozen in liquid nitrogen and stored at − 70°C for the measurement of NO synthase activity.

Experimental protocol

To assess autoregulation in the kidney we injected pentobarbitone sodium (30 mg kg^{-1}, i.v. bolus, $n = 21$). This produced a transient fall in MAP ($\Delta MAP = 20 \pm 2$ mmHg), but no changes in RBF (-0.2 ± 0.2 ml min^{-1}) showing that the surgical procedures did not affect the autoregulatory adjustments of the renal circulation.

To assess the reponse of the renal vascular bed to endothelium-dependent vasodilators, acetylcholine (ACh, 10 μg kg^{-1} min^{-1}, i.v. for 5 min; infusion rate 0.1 ml min^{-1}; $n = 9$) was infused before and 1 h after occlusion of the renal artery. In some experiments, ACh was given before and after, an intravenous infusion of L-arginine (L-Arg, 20 mg kg^{-1} min^{-1} for 15 min; $n = 4$).

To investigate the role of basal release of NO in the regulation of RBF following ischaemic injury of the renal vascular bed, L-NAME (30 μg kg^{-1} min^{-1}, i.v., $n = 5$), an inhibitor of both constitutive and inducible isoforms of NO synthase (Moore *et al.*, 1990; Thiemermann *et al.*, 1990; Hecker *et al.*, 1990), was infused (at 1.5 ml h^{-1}) starting 30 min after the onset of ischaemia and continued throughout the reperfusion period. In a separate set of experiments, the effect of L-NAME (30 μg kg^{-1} min^{-1}, i.v., $n = 4$) was investigated in SOP-animals. The effects of L-Arg (1 or 3 mg kg^{-1} min^{-1}, $n = 5$ and $n = 4$, respectively) or D-Arg (1 mg kg^{-1} min^{-1}, $n = 6$) were measured on RBF and RVR by continuous infusions starting 30 min after the onset of ischaemia.

In other experiments, animals were pretreated with dexamethasone (3 mg kg^{-1}; i.v. after surgical procedure) to prevent induction of NO synthase. The concentration of dexamethasone used was sufficient to inhibit the synthesis of NO synthase in response to cytokines *in vitro* (Radomski *et al.*, 1990) and *in vivo* (Knowles *et al.*, 1990).

To investigate the potential contribution of prostaglandins to the maintenance of RBF after I/R, animals received indomethacin (5 mg kg^{-1}, i.v. bolus, $n = 3$) 15 min after surgical procedures and were allowed to stabilize for 30 min. Another group of animals ($n = 4$) were treated with indomethacin (above protocol) and L-NAME (30 μg kg^{-1} min^{-1}, i.v.) to investigate the relative contribution of prostanoids and NO to the maintenance of RBF after renal ischaemia.

Measurement of NO synthase activity

NO synthase activity was measured in both ischaemic and non-ischaemic kidneys obtained from control and sham-operated rats. To be sure that the measurements were sensitive enough to measure the induction of NO synthase (positive control), a separate group of animals was subjected to injection of lipopolysaccharide (5 mg kg^{-1}, i.p., $n = 3$), a well known inducer of NO synthase activity *in vitro* and *in vivo* (Moncada *et al.*, 1991; Thiemermann, 1991; Stuehr & Griffith, 1992). Six hours after endotoxaemia, the kidneys were removed, frozen in liquid nitrogen and stored at − 70°C.

The frozen kidneys were homogenized on ice in a buffer: Tris/HCl (50 mM); EDTA (0.1 mM); EGTA (0.1 mM); 2-mercaptoethanol (12 mM) and phenylmethylsulphonyl fluoride (1 mM, pH 7.4) with an Ultra-turrax T25 homogenizer. Conversion of [^{3}H]-L-arginine to [^{3}H]-L-citrulline was measured in the homogenates (Bredt & Snyder, 1990). Briefly, 50 μl of tissue homogenate (approximately 100 μg protein) was incubated in the presence of L-arginine and [^{3}H]-L-arginine (10 mM, 5000 Bq/tube), NADPH (1 mM), calmodulin (30 mM), valine (50 mM), tetrahydrobiopterin (5 μM) and calcium (2 mM) for 20 min in HEPES buffer (pH 7.5). The reaction was stopped by the addition of HEPES buffer (pH 5.5) containing EGTA (2 mM) and EDTA (2 mM). The reaction mixture was applied to Dowex 50W (Na form) columns (Sigma Chemical Co., Poole, Dorset) and the eluted [^{3}H]-L-citrulline

activity was measured by scintillation counting (Beckman, 2 S 3801). Experiments performed in the absence of NADPH determined the extent of [^{3}H]-L-citrulline formation independent of a specific NO synthase activity. In addition, experiments in the presence of NADPH and calcium or in the presence of NADPH without calcium determined the calcium dependent and the calcium independent (i.e. total and inducible) NO synthase activity, respectively (Mitchell *et al.*, 1991; Salter *et al.*, 1991).

Materials

Trapanal (thiopentone sodium) was obtained from B.Y.K. Gulden (Konstanz, Germany) and Sagatal (pentobarbitone sodium) from RMB Animal Health Ltd. (Dagenham) acetylcholine chloride, dexamethasone 21-phosphate disodium salt, indomethacin, calcium, calmodulin, valine, L-arginine hydrochloride, D-arginine hydrochloride and N^G-nitro-L-arginine methyl ester were purchased from Sigma Chemical Co. (Poole, Dorset). All compounds were dissolved in 0.9% saline except for indomethacin which was prepared as a 5 mg kg^{-1} solution in 5% w/v sodium bicarbonate. [^{3}H]-L-arginine was obtained from Amersham (Buckinghamshire) and tetrahydrobiopterin from Dr B. Schircks Laboratories (Jona, Switzerland).

Statistical analysis

All values in the figures and text are expressed as mean ± s.e.mean of n observations. Statistical comparisons of differences within the same animal were made by Student's t test for paired determinations; comparisons of differences between groups of animals were made by Student's t test for unpaired determination. A P value of less than 0.05 was considered significant.

Results

Impairment of endothelium-dependent vasodilator responses after ischaemia-reperfusion injury of the renal vascular bed

After the stabilization period, baseline values were 12 ± 0.3 ml min^{-1} for RBF, 123 ± 2 mmHg for MAP and 11 ± 0.3 mmHg min ml^{-1} for RVR (n = 59). No significant changes in any of those haemodynamic parameters were observed in SOP animals (n = 4) during the 6 h experimental period (not shown for MAP and RVR; see Figure 1 for RBF). After 60 min of total cessation of RBF (ischaemic period), RBF recovered to 9 ± 1 ml min^{-1} (representing 69 ± 5% of the pre-occlusion value) within 30 min of reperfusion (n = 6). However, RBF remained significantly smaller than the pre-ischaemic level during the subsequent 2 or 6 h reperfusion period (Figure 1). RVR was significantly increased to 16 ± 2 mmHg min ml^{-1} after 30 min of reperfusion compared to the pre-ischaemic level, while no change was observed in MAP (MAP at 30, 60, 120 and 360 min of reperfusion: 130 ± 3, 128 ± 2, 126 ± 2, 127 ± 5 mmHg, respectively). Moreover, RVR remained significantly elevated when compared to either pre-occlusion values in the same animal or with SOP.

Before I/R injury, an infusion of ACh (10 μg kg^{-1} for 5 min) produced a significant (P<0.01) fall in MAP amounting to 13 ± 2 mmHg (10 ± 1%) (n = 9). In spite of this decrease of MAP, RBF was increased by 3 ± 0.3 ml min^{-1} (22 ± 2%; P<0.01) (Figure 2) and RVR was substantially decreased by 3 ± 0.5 mmHg min ml^{-1} (26 ± 2%; P<0.01).

After I/R injury, the fall in MAP in response to ACh was unchanged (ΔMAP: 13 ± 2 mmHg; 9 ± 1%, P<0.01). In contrast, the endothelium-dependent vasodilator responses to ACh in the renal vascular bed after I/R were impaired (ΔRBF: 1 ± 0.3 ml min^{-1}, 11 ± 3%, P<0.01; ΔRVR: 3 ± 0.3 mmHg min ml^{-1}, 18 ± 3%, P<0.01). When compared to pre-ischaemic control responses, the ACh-induced increase in RBF measured either as absolute value (3 ± 0.3 versus 1 ± 0.3 ml min^{-1}, P<0.01) or percentage change (22 ± 2 versus 9 ± 1%, P<0.01) was significantly reduced after I/R injury (Figure 2). When analysed as absolute value, the ACh-induced fall in RVR was not altered by I/R injury (3 ± 0.5 versus 3 ± 0.3 mmHg min ml^{-1}, P<0.05), whereas the percentage fall in RVR was significantly reduced by I/R injury (control: 26 ± 2%; I/R; 18 ± 3%, P<0.01).

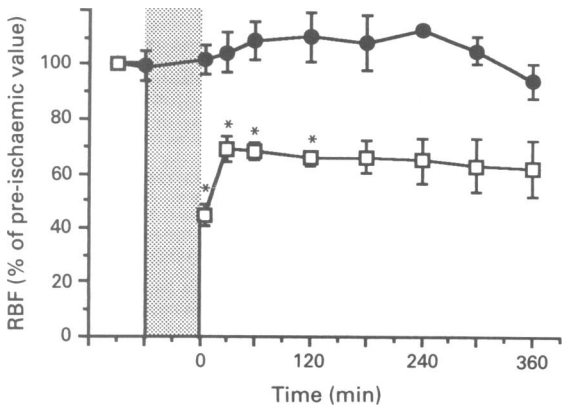

Figure 1 Changes in renal blood flow (RBF, percentage of pre-occlusion value) in sham-operated animals (SOP, ●, n = 4) and in animals submitted to 1 h ischaemia (stippled area) followed by 6 h of reperfusion (□, n = 6) of the renal vascular bed. Results are expressed as mean ± s.e.mean; *P<0.05 when compared with pre-occlusion value by Student's t test for paired determinations

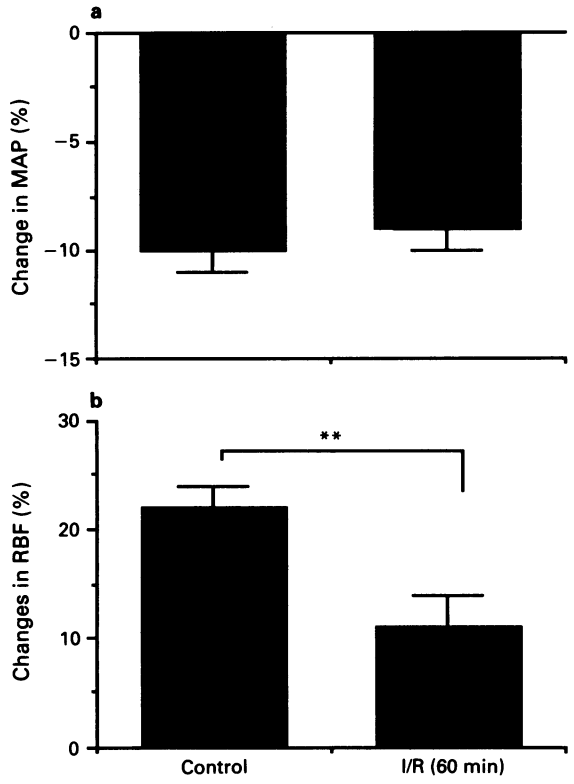

Figure 2 Ischaemia and reperfusion impairs the vasodilator responses to acetylcholine (ACh) in the renal vascular bed. Depicted are the effect of the endothelium-dependent vasodilator ACh on (a) mean arterial blood pressure (MAP) and (b) renal blood flow (RBF) before (control) and after 1 h of ischaemia followed by 60 min of reperfusion of the rat kidney *in vivo*. Data are expressed as mean ± s.e.mean of 9 observations. *P<0.05, **P<0.01 when compared by a Student's t test for paired determinations

Before renal artery occlusion, L-Arg (20 mg kg^{-1} min^{-1} for 15 min) produced a slight, but significant increase in RBF (1 ± 0.3 ml min^{-1}, 10 ± 1%, $P<0.05$). L-Arg had no effect on MAP, so that RVR decreased by 10 ± 1% ($P<0.05$). However, L-Arg did not affect either the systemic or renal vasodilator effect of ACh ($n=4$). After I/R injury, L-Arg produced the same increase in RBF (1 ± 0.2 ml min^{-1}, 10 ± 2%), but failed to restore the ischaemia-induced impairment of the vasodilator response to ACh (Figure 3).

Importance of the generation of NO from the constitutive, but not the inducible NO synthase for the maintenance of RBF after I/R injury of the renal vascular bed

Infusion of L-NAME (30 μg kg^{-1} min^{-1}) significantly attenuated the recovery of RBF after I/R. After 2 h of reperfusion, RBF was reduced to 3 ± 0.5 ml min^{-1} ($n=5$, $P<0.01$) representing 29 ± 3% of the pre-occlusion value (Figure 4a). L-NAME produced a significant increase in MAP (ΔMAP: 24 ± 6 mmHg) and MAP remained significantly elevated throughout the reperfusion period (Figure 4a). Both the systemic and renal effects of L-NAME resulted in a substantial increase in RVR to 46 ± 1 mmHg min ml^{-1} ($P<0.01$) at the end of experiment (Figure 4b). L-NAME produced a similar degree of fall in RBF from 11 ± 1 ml min^{-1} to 4 ± 1 ml min^{-1} after 2 h of infusion in SOP animals ($P<0.01$, Figure 4). When compared to L-NAME, indomethacin (5 mg kg^{-1}, i.v., 30 min before renal artery occlusion, $n=3$) had no effect on RBF but caused a significant rise in RVR after I/R injury (Table 1). However,

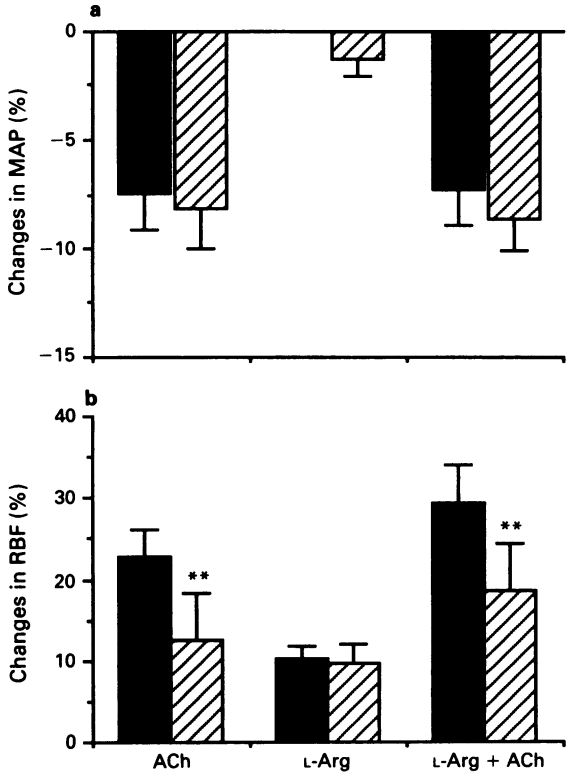

Figure 3 Effect of infusion of L-arginine (L-Arg) on the ischaemia-induced impairment of the vasodilator response to acetylcholine (ACh) in the rat. Depicted are changes in (a) mean arterial blood pressure (MAP) and (b) renal blood flow (RBF) induced by ACh (10 μg kg^{-1} min^{-1}, i.v. for 5 min; $n=4$), L-Arg (20 mg kg^{-1} min^{-1}, for 15 min; $n=4$) and L-Arg plus ACh before ischaemia (solid columns) and after 1 h of ischaemia followed by 1 h of reperfusion (hatched columns). Results are expressed as mean ± s.e.mean of 4 observations. *$P<0.05$ when compared by Student's t test for paired determinations

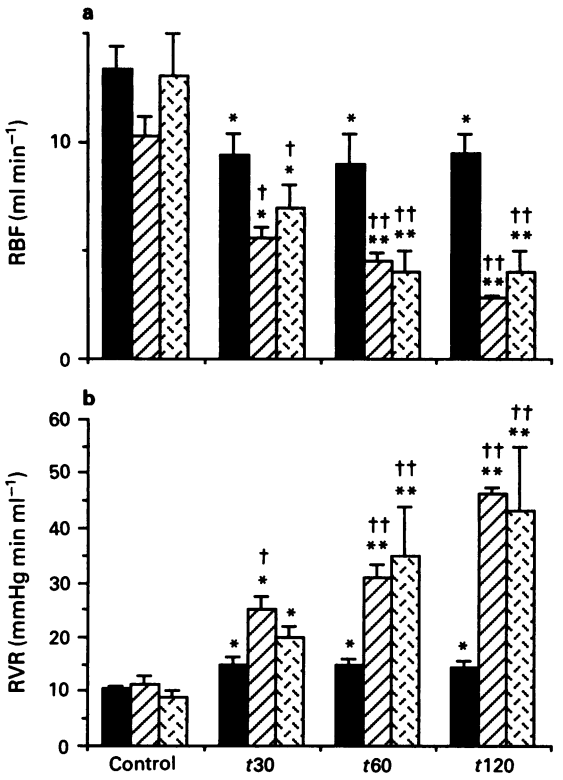

Figure 4 Inhibition of NO formation significantly reduced the recovery of RBF after ischaemia reperfusion injury of the renal vascular bed. Depicted are the changes in (a) renal blood flow (RBF) and (b) renal vascular resistance (RVR) in animals subjected to 60 min ischaemia followed by 120 min of reperfusion (t 30, t 60, t 120) in the absence (control, solid columns, $n=6$), or in the presence of N^{G}-nitro-L-arginine methyl ester (L-NAME, 30 μg kg^{-1} min^{-1}, i.v., hatched columns, $n=5$). In addition, sham-operated animals were treated with L-NAME (30 μg kg^{-1} min^{-1}, i.v., stippled columns, $n=5$). Data are expressed as mean ± s.e.mean. *$P<0.05$, **$P<0.01$ when compared by Student's t test for paired determination; †$P<0.05$, ††$P<0.01$ when compared by Student's t test for unpaired determinations

indomethacin did not affect the changes in RVR or RBF produced by L-NAME in rats subjected to I/R injury (Table 1).

Dexamethasone (3 mg kg^{-1}, i.v., 60 min prior to I/R), an inhibitor of the induction of NO synthase (Radomski *et al.*, 1990; Knowles *et al.*, 1990), had no effect on either RBF or RVR in rats subjected to ischaemia followed by either 2 ($n=6$) or 6 h ($n=3$) of reperfusion (Table 1). Moreover, no calcium-independent (inducible) NO synthase activity was detected in kidney homogenates obtained from either SOP rats or from animals subjected to I/R for 2 ($n=4$) or 6 h ($n=3$). However a calcium-independent NO synthesis (19 ± 2 pmol mg^{-1} 20 min^{-1}) occurred in the kidney homogenates obtained from rats treated with LPS (positive control, $n=3$).

L-Arg (1 or 3 mg kg^{-1} min^{-1}) or D-Arg (1 mg kg^{-1} min^{-1}) did not cause any change in MAP. Moreover, L-Arg or D-Arg had no effect on either the changes in RBF or RVR associated with I/R of the renal vascular bed (Table 1).

Discussion

This study demonstrates that formation of endogenous NO is important not only for the maintenance of normal RBF, but also for the recovery and maintenance of RBF after I/R injury of the renal vascular bed. The model of I/R injury of the kidney used in this study results in haemodynamic alterations within the renal vasculature including a rise in RVR, a

Table 1 Changes in (**A**) renal blood flow (RBF) and (**B**) renal vascular resistance (RVR) in rats subjected to 60 min ischaemia followed by 120 min of reperfusion

		Before	*30 min*	*60 min*	*120 min*
A	*RBF*	(ml min)			
	I/R	13 ± 1	9 ± 1*	9 ± 1*	9 ± 1*
	L-NAME	10 ± 1	6 ± 1*†	5 ± 1**††	3 ± 1**††
	Indo + L-NAME	10 ± 1	7 ± 1*	5 ± 1*†	4 ± 1**††
	Indo	10 ± 1	7 ± 1*	7 ± 1*	7 ± 1*
	Dexamethasone	13 ± 1	10 ± 1*	10 ± 1*	10 ± 1*
	L-Arg (1 mg)	11 ± 1	9 ± 1*	8 ± 1*	9 ± 1*
	L-Arg (3 mg)	11 ± 1	7 ± 1*	8 ± 1*	8 ± 1*
	D-Arg (1 mg)	13 ± 2	9 ± 1*	8 ± 1*	8 ± 1*
B	*RVR*	(mmHg min ml^{-1})			
	I/R	11 ± 0.5	16 ± 2*	15.7 ± 1*	15 ± 1*
	L-NAME	11.3 ± 1	25 ± 2*†	31 ± 2**††	46 ± 1**††
	Indo + L-NAME	9 ± 1	25 ± 9*	26 ± 6*†	37 ± 6*††
	Indo	12 ± 1	22 ± 2*†	21 ± 2*†	20 ± 2*†
	Dexamethasone	9 ± 1	13 ± 1*	13 ± 1*	13 ± 1*
	L-Arg (1 mg)	11 ± 1	14 ± 2*	14 ± 2*	14 ± 2*
	L-Arg (3 mg)	12 ± 1	20 ± 3*	19 ± 3*	18 ± 3*
	D-Arg (1 mg)	10 ± 1	16 ± 2*	16 ± 2*	16 ± 2*

Different groups of animals received vehicle (I/R, $n = 6$), (30 $\mu g\ kg^{-1}\ min^{-1}$, i.v., $n = 5$), indomethacin (Indo, 5 $mg\ kg^{-1}$, i.v. bolus, $n = 3$), indomethacin and N^G-nitro-L-arginine methyl ester (L-NAME) (Indo + L-NAME, $n = 4$), dexamethasone (3 $mg\ kg^{-1}$; i.v., $n = 6$), L-arginine (1 or 3 $mg\ kg^{-1}\ min^{-1}$), or D-arginine (1 $mg\ kg^{-1}$). Data are expressed as mean ± s.e.mean.
*$P<0.05$, **$P<0.01$ when compared by Student's t test for paired determinations; †$P<0.05$, ††$P<0.01$ when compared by Student's t test for unpaired determinations.

fall in RBF, a loss of autoregulation (Kashgarian *et al.*, 1976; Riley, 1978) and an impairment of the response to endothelium-dependent vasodilators (Conger *et al.*, 1991). In the isolated erythrocyte-perfused kidney of the rat, 25 min of ischaemia followed by reperfusion is associated with a marked increase in RVR (Lieberthal *et al.*, 1989). In this model, inhibition of NO formation with two non-selective inhibitors (methylene blue and gossypol) resulted in no further increase in RVR after I/R injury *in vitro* indicating an ischaemia-induced complete loss of the release of NO. However, we demonstrate here that in the anaesthetized rat *in vivo*, inhibition of NO formation with L-NAME, a selective and potent inhibitor of NO formation both *in vitro* and *in vivo* (Moore *et al.*, 1990; Thiemermann *et al.*, 1990; Hecker *et al.*, 1990) is associated with a marked reduction in RBF and a further enhancement of RVR (Figure 4), indicating that NO release contributes to the recovery of RBF after I/R. The reduction in RBF and the increase in RVR obtained after maximal inhibition of NO formation with L-NAME was not different in SOP-animals or rats subjected to I/R injury indicating that I/R is not associated with a substantial impairment of the basal release of NO.

The hypothesis that NO is released by the constitutive, but not inducible NO synthase, is supported by the findings that the recovery of RBF after I/R injury is largely inhibited by L-NAME (Figure 4), an inhibitor of both constitutive and inducible NO synthase, but is not affected by dexamethasone (Table 1), an inhibitor of the induction of NO synthase (Knowles *et al.*, 1990; Radomski *et al.*, 1990). Moreover, no inducible NO synthase was detected after 60 min of renal artery occlusion followed by either 2 or 6 h of reperfusion, even though cytokines, such as tumour-necrosis factor, are released by ischaemia reperfusion injury (Ascer *et al.*, 1992; Holtz & Goetz, 1992). The lack of activity of an inducible NO synthase after I/R was not due to a lack of sensitivity of the NO synthase assay employed, for i.p. injection of LPS, which causes induction of NO synthase, resulted in a significant increase in a calcium-independent NO synthase activity.

The hypothesis that I/R is associated with a reduced formation of NO is based on findings demonstrating an ischaemia-induced impairment of the response to the endothelium-dependent vasodilator ACh (Conger *et al.*, 1991; Sobey *et al.*, 1992). However, the present study demonstrates that an ischaemia-induced impairment of the vasodilator responses to ACh is not necessarily associated with a substantial reduction or even loss of the basal release of NO. Ischaemic injury of the myocardium is also associated with a reduced vasodilator response to ACh (Sobey *et al.*, 1992) despite a normal formation of NO (McMurdo *et al.*, 1992). Although hypoxia impairs endothelium-dependent relaxation to ACh in the pulmonary vascular bed (Warren *et al.*, 1989), the basal release of NO after unilateral hypoxia in the pulmonary vasculature of the rabbit is sufficient to oppose hypoxic vasoconstriction resulting in a mismatching of ventilation to perfusion (Sprague *et al.*, 1992). At present the mechanism of the ischaemia-induced impairment of the responses to ACh is unclear. However, a reduced availability of L-Arg (Radermacher *et al.*, 1991) is unlikely to account for this impairment, for infusion of L-Arg did not restore the responses to ACh. Interestingly, more evidence is emerging indicating that the mechanisms that regulate basal and receptor stimulated release under physiological conditions are different (Hecker *et al.*, 1992; MacArthur *et al.*, 1993).

In addition to NO, vasodilator prostanoids, presumably prostacyclin, contribute to the maintenance of RBF following I/R injury of the kidney, as indomethacin caused a rise in RVR which was significant throughout the 120 min reperfusion period. It is well known that cyclo-oxygenase inhibitors have little or no effect on normal RBF, but do reduce RBF when the renal circulation is compromised (Oliver *et al.*, 1981; Stoff & Clive, 1983). Thus, endogenous release of cyclo-oxygenase products is a reserve mechanism, not increasing normal RBF but helping to maintain a damaged circulation. Endogenous release of NO, however, is important in maintaining normal RBF, for synthesis inhibitors substantially reduce it. The fact that NO synthase inhibitors also reduce RBF when the blood flow has been compromised by I/R injury shows that the NO release mechanism is still functional, although there is no evidence that it has been increased.

What then accounts for the I/R-induced increase in RVR observed in this study? Clearly, I/R injury is associated with the formation of vasoconstrictor agents, such as angiotensin II (Stein *et al.*, 1978) or endothelin-1 (Kon & Badr, 1991). For instance, infusion of anti-endothelin-1 antibodies into the renal artery largely attentuates the rise in RVR following I/R in the anaesthetized rat (Kon & Badr, 1991). Hence, it is

possible that an increased formation of endogenous vasoconstrictors such as endothelin-1 contributes to the I/R-induced impairment of RBF, while the formation of endothelium-derived vasodilator autacoids serve as an endogenous defense mechanism to maintain a sufficient RBF.

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