

Original Article

L-Arginine reduces tubular cell injury in acute post-ischaemic renal failure

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Abstract

Background. The pathophysiology of renal ischaemia, resulting in tubular cell injury and leading to acute renal failure (ARF), remains unclear. An ever-increasing number of investigations focus on a possible role of nitric oxide (NO) in regulating circulation during ARF. In this context, we investigated the influence of chronic stimulation or inhibition of NO synthesis, or both, on haemodynamic parameters, histology and plasma renin activity (PRA) after ischaemia–reperfusion injury of rat kidneys.

Methods. Experiments were performed on adult, male Wistar rats. Before induction of ARF, a group of animals was treated with a NO synthesis inhibitor (L-NAME) and another group was treated with a precursor of NO synthesis (L-arginine). The animals received those substances for 4 weeks. Control groups received the same amount of tap water for 4 or 8 weeks and were divided into groups with ARF (4 weeks–ARF group and 8 weeks–ARF group) and a sham-operated group. Another group of rats was treated first with L-NAME and then with L-arginine in their drinking water, for 4 weeks for each of these two substances. All parameters were evaluated 24 h after the induction of ischaemic ARF or the sham operation.

Results. Our results show that such long-term stimulation of NO release by L-arginine improved renal haemodynamics in the ischaemic form of ARF. Renal blood flow (RBF) increased by 96% in the L-arginine-treated rats with ARF compared with the group with ARF alone. Inhibition of NO synthesis worsens renal haemodynamics after ARF. However, this aggravation can be reversed by L-arginine. The rate of water reabsorption was reduced in all groups with ARF, but this reduction was least in the group treated with L-arginine. The rate of Na⁺ reabsorption was reduced in all groups 24 h after renal ischaemia, but a significant

decrease was observed after the inhibition of NO synthesis. Histological examination of the kidney specimens showed that morphological changes were least in the rats treated with L-arginine, when compared with all other groups with ARF. Nevertheless, the lesions were most prominent in the L-NAME + ARF group. In this group, the areas of corticomedullary necrosis were more widespread in comparison with other groups, especially the L-arginine group where only swelling of the proximal tubular cells was observed. Treatment with L-NAME was not accompanied by any significant alteration in the plasma concentration of angiotensin I (ANG I), while in the group treated with L-arginine ANG I had a tendency to decrease.

Conclusions. Acute post-ischaemic renal failure may be alleviated by administering the NO substrate (L-arginine). NO acts cytoprotectively on tubular epithelial cells in ischaemia–reperfusion injury of rat kidney. Evidence of this comes from both histopathological findings and increased tubular water and sodium reabsorption. However, inhibition of NO synthesis (provoked by L-NAME) worsens renal haemodynamics and aggravates morphological changes after ARF. These aggravations can, however, be reversed by L-arginine.

Key words: acute renal failure; angiotensin I; haemodynamics; nitric oxide; renal morphology

Introduction

Nitric oxide (NO), an endothelium-derived relaxing factor, is produced mainly from L-arginine, which is mediated by NO synthases. Previous reports have shown that NO plays an important role in the regulation of systemic and renal haemodynamics [1,2]. NO may have a role to play in the pathophysiological mechanisms in different renal diseases [3,4], but the mechanism of its action on the circulation in acute

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renal failure (ARF) has yet to be elucidated. In addition, the effects of NO on tubular morphology and function are even less clearly understood. Hence, studies on the role of NO synthesis in the regulation of the renal blood flow and function are of high pathophysiological and therapeutic relevance. Besides, decreased synthesis or action of NO has been implicated in some other diseases, such as hypertension, hypercholesterolaemia, diabetes or atherosclerosis [5]. This study was designed to investigate whether chronic manipulation of the NO system (stimulation or inhibition of NO synthesis, or both) influences ischaemia-reperfusion injury of rat kidneys, as seen through its haemodynamic and histopathological parameters.

Evidence is also emerging that NO contributes to the regulation of renin secretion. Therefore, this was among our topics for investigation. A functional interaction between NO and the renin-angiotensin system might be expected since the effects of NO on systemic and renal haemodynamics appear to be opposite to those of angiotensin II. It has been shown [6,7] that the plasma renin activity is increased in the initial stages of ARF. In this context, we examined whether a long-term manipulation of the NO system changed the plasma renin activity (PRA) in the early stage of post-ischaemic ARF.

Subjects and methods

Experiments were performed on adult male Wistar rats (200–250 g). All animals were given standard food for laboratory rats (Veterinarski zavod, Zemun, Yugoslavia). Two separate baseline determinations of body mass and systolic blood pressure were made in all rats over a period of 2 weeks. Using a tail-cuff, pneumatic pulse detector and a direct recorder (Physiograph Four, Narco Bio-System, Houston, TX, USA) systolic blood pressure was measured indirectly. The animals were divided into six groups (as follows) according to the drugs they were given during the treatment period (of 4 or 8 weeks). All animals were eventually subjected to ARF, with the exception of the sham-operated rats (see Fig. 1A outlining the experiment).

Group 1:

sham-operated rats ($n=18$) received tap water—100 ml/kg body mass (b.m.) for 4 weeks.

Group 2:

control rats ($n=22$) received the same amount of tap water for 4 weeks and then ARF was induced (4 weeks-ARF group).

Group 3:

L-NAME (N^G -L-arginine methyl ester; 10 mg/kg b.m./day), a NO synthesis inhibitor, was given in the drinking water for 4 weeks (L-NAME+ARF group, $n=16$).

Group 4:

L-arginine (2 g/kg b.m./day), a substrate for NO was given in the drinking water for 4 weeks (L-Arg+ARF group, $n=16$).

Group 5:

rats ($n=16$) were treated first with L-NAME (10 mg/kg b.m./day) and then with L-arginine (2 g/kg b.m./day) in

the drinking water, for 4 weeks for each of these two substances (L-NAME+L-Arg+ARF group).

Group 6:

control rats ($n=16$) for the previous group of animals. They received tap water for 8 weeks and then were subjected to ARF (8 weeks-ARF group).

Blood pressure and body mass were measured once a week during the 4- and 8-week course of treatment respectively. Before the induction of ischaemic ARF, endothelium-dependent NO-mediated vasodilatation was examined using acetylcholine (Ach). Animals were anaesthetized using intraperitoneal sodium pentobarbital (Nembutal), 35 mg/kg b.m. The femoral artery (for blood pressure measurement) and femoral vein were cannulated and Ach was administered as three i.v. boluses (0.2 μ g/kg b.m., 0.4 μ g/kg b.m. and 0.6 μ g/kg b.m.). Boluses were separated by time intervals allowing mean arterial pressure (MAP) to return to the baseline value. MAP was measured with a pressure transducer and recorded on a direct writing recorder. Vasodilative response to Ach was calculated for each animal as a percentage of the MAP decline from its baseline value. After a 30-min wash-out interval and recovering of MAP, ARF was induced. The right kidney was removed, and the left renal artery was gently separated from the renal vein and clamped with an atraumatic clamp for 45 min. The sham-operated rats were right nephrectomized. Immediately after surgery all rats were placed in individual metabolic cages and urine was collected for the next 24 h. On the last experimental day the treated animals continued to receive the same amount of the L-NAME or L-arginine as they did previously. The water and thus the drug intake were measured.

Haemodynamic measurements 24 h after reperfusion

Haemodynamic parameters were measured after urine collection in anesthetized (35 mg/kg sodium pentobarbital) rats: sham-operated rats ($n=10$), 4 weeks-ARF group ($n=14$), L-NAME+ARF group ($n=8$), L-Arg+ARF group ($n=8$), L-NAME+L-Arg+ARF group ($n=8$) and the respective control animals, 8 weeks-ARF group ($n=8$). Blood pressure was measured directly through a femoral artery catheter (PE-50, Clay-Adams, Parsippany, NJ, USA), using a low-volume displacement transducer (P23 Db; Statham, Oxnard, CA, USA) and a direct writing recorder. MAP was obtained by electronic integration. Cardiac output (CO) was determined using a previously described [8] modification of Coleman's application of the dye dilution technique [9]. Total peripheral resistance (TPR) was calculated from MAP and CO (assuming that mean right atrial pressure is zero). For the blood-flow measurement the left renal artery was gently separated. An ultrasonic flow probe (1RB, internal diameter=1 mm) was placed around the artery to measure the total renal blood flow (RBF), using a Transonic T106 Small Animal Flowmeter (Transonic System Inc., Ithaca, NY, USA). Renal vascular resistance was calculated by dividing MAP by renal blood flow.

Biochemical measurements and morphology

Urinary and plasma creatinine concentrations were determined using a Beckman 42 spectrophotometer. Concentrations of sodium (Na^+) and potassium (K^+) in the plasma and urine were measured using a IL 943-flame photometer (Instrumentation Laboratory, Milan, Italy). A standard formula was used to calculate creatinine clearance. Fractional excretion of electrolytes was calculated as a percentage of

creatinine clearance. Reabsorption rates of Na^+ and water at tubular sites were calculated using the formulae quoted in Kusaka *et al.* [10].

Histological examination of the left kidney was also made 24 h after reperfusion. The renal tissue was fixed in 10% buffered formalin solution. Later, the kidney was dehydrated in alcohol, blocked in paraffin wax and 5- μm -thick sections were cut and stained by periodic acid-Schiff reaction. Light microscopy (LM) evaluations were made so those acute tubular lesions were graded on a scale from 0 to 4+ according to the degree of severity:

- 0 = normal tubular cells,
- 1+ = loss of luminal membrane or brush borders,
- 2+ = swelling and vacuolization of cells,
- 3+ = separation of cells from the basement membrane,
- 4+ = as 3+ with nude basement membrane.

The severity of congestion, i.e. the accumulation of red blood cells in glomeruli, peritubular capillaries and intrarenal veins, was graded on a scale from 1+ to 3+ as described by Mandal *et al.* [11]. The presence of tubular dilatation, cast formations, mononuclear infiltration and interstitial oedema was noted with 1, and their absence with 0. Vascular changes (in arterial vessel walls) were graded according to a scoring system proposed by Mandal and co-workers [12]. The sum of these changes was the histopathological score for comparison between the groups.

Two independent investigators made histological evaluations; if their assessment disagreed, consensus was reached by discussion, whereas for the histopathological score the mean value was calculated for each group.

In the remaining rats from each group ($n=8$), PRA levels were measured. To determine PRA, animals were guillotined and blood samples collected into chilled, siliconized, centrifuge tubes containing Na_2EDTA (2 mg/ml). REN-CT2 angiotensin I radioimmunoassay (RIA) kit (CIS Bio International, France), were used in radioimmunoassay procedures.

Statistical analyses

Results are expressed as mean \pm SEM. One-way analysis of variance (ANOVA) was applied. When the ANOVA results were significant, Bonferroni's *t*-test was used to determine the level of significance where a *P*-value < 0.05 was considered to be significant (*Primer of Biostatistics*, by Stanton A. Glanz).

Results

Treatment with L-NAME, L-arginine or both had no influence on body mass. The systolic blood pressure was significantly higher 1 week after the beginning of treatment with L-NAME and was still elevated by the end of the experiment, whereas in the groups not receiving NO synthesis inhibitor systolic blood pressure remained unchanged (Figure 1B). In the L-arginine-treated rats, systolic blood pressure was slightly lowered only 4 weeks after the beginning of the treatment. In the group treated with both L-NAME and L-

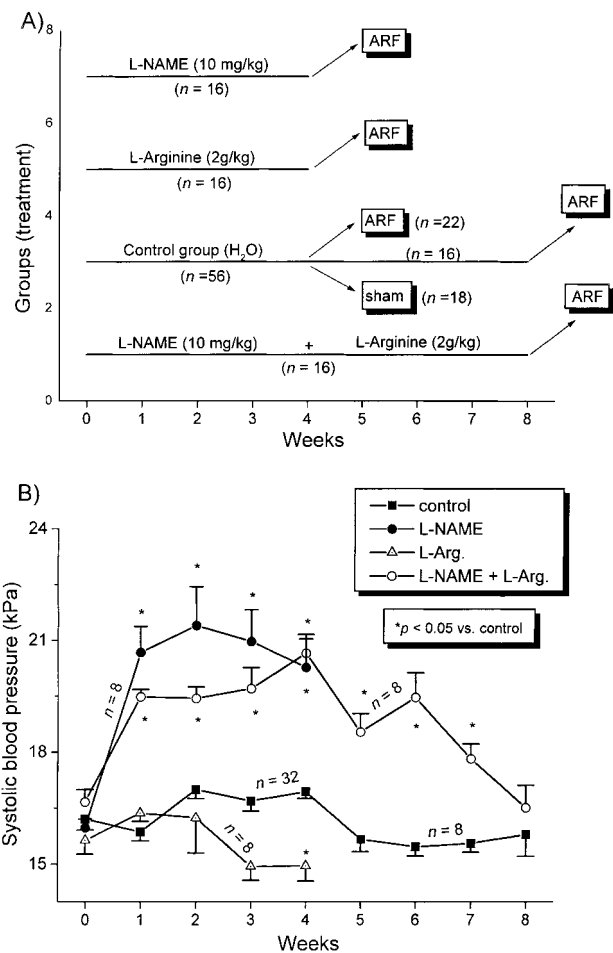


Fig. 1. (A) Experiment outlined; (B) systolic blood pressure during 4 or 8 weeks before induction of ARF in: control rats, L-NAME treated rats, L-arginine treated rats and rats treated first with L-NAME and then with L-arginine.

arginine, systolic blood pressure was significantly higher during the treatment with L-NAME and then decreased slowly in the period when the rats received L-arginine reaching the control level at the end of the treatment (Figure 1B).

Ach had a vasorelaxant effect. The effect was generally dose dependent, except for the greatest dose in the control group and the group pretreated with L-arginine. It can be clearly seen in Fig. 2A that the vasorelaxant effect in rats pretreated with L-NAME is considerably less than (and significantly different from) the effect in all other groups. However, in rats pretreated first with L-NAME and then with L-arginine, the effect is clearly pronounced and strongly dose dependent.

Blood pressure during ARF-induction is shown in Fig. 2B. MAPs before and after clamping are not significantly different. After the moment of clamping the pressure decreased insignificantly in the rats of all groups, and above all in rats pre-treated with L-NAME.

The systemic haemodynamic parameters, SHP (MAP, CO and TVR) for all groups are summarized in Figure 3. Several points are important to note:

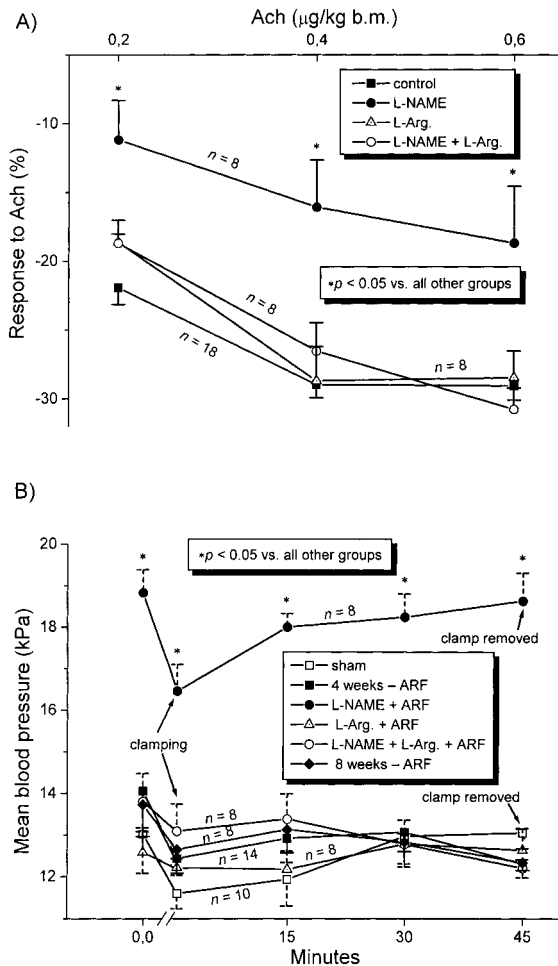


Fig. 2. (A) Concentration-response to acetylcholine (Ach) in: control rats, L-NAME treated rats, L-arginine treated rats and rats treated first with L-NAME and then with L-arginine. (B) Mean blood pressure during ARF-induction in: sham-operated rats, control rats with acute renal failure (4 weeks-ARF), L-NAME-treated ARF rats, L-arginine treated ARF rats, rats treated first with L-NAME and then with L-Arg (L-NAME+L-Arg+ARF group), and in rats of the (8 weeks-ARF) group, serving as a control for the last group.

(i) the SHP were not altered 24 h after reperfusion in either control group with ARF when compared with the sham-operated rats; (ii) in neither the L-Arg + ARF group nor in the L-NAME + L-Arg + ARF group was the SHP improved; (iii) treatment with L-NAME noticeably increased the MAP and TVR in comparison with all other groups.

Changes in haemodynamic parameters in the renal artery are shown in Figure 4. After the induction of ARF, in both control ARF groups (4 weeks-ARF and 8 weeks-ARF) the RBF was reduced by 35% compared with the sham-operated animals, but the difference was not significant. The renal vascular resistance (RVR) was slightly higher after inducing ARF in both control ARF groups. L-NAME-induced inhibition of NO synthesis caused a significant decrease of the RBF in those animals, compared with that of sham-operated rats; and a significant increase of the RVR, in comparison with all other groups. Stimulation of NO synthesis by

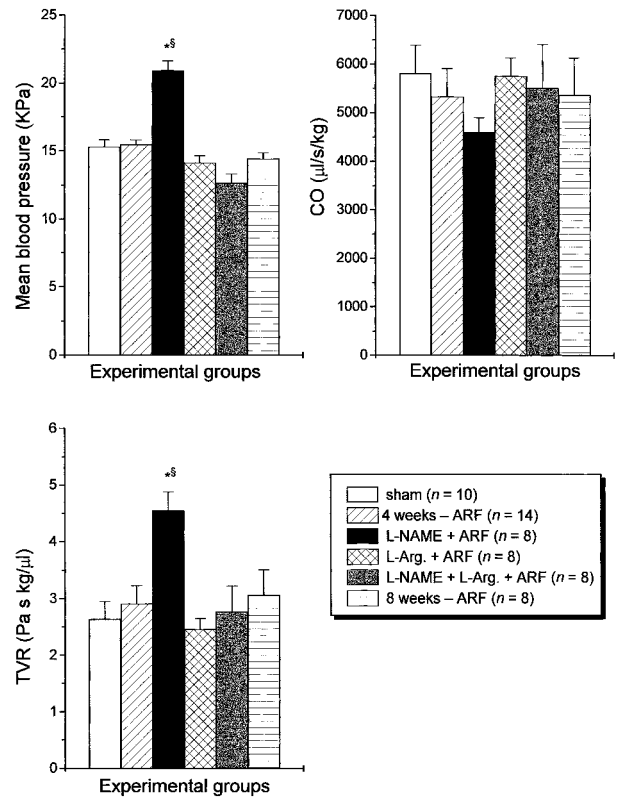


Fig. 3. Mean blood pressure, cardiac output (CO) and total vascular resistance (TVR) in: sham-operated rats, control rats with acute renal failure (4 weeks-ARF), L-NAME-treated ARF rats, L-arginine treated ARF rats, rats treated first with L-NAME and then with L-Arg (L-NAME + L-Arg + ARF group), and in rats of the (8 weeks-ARF) group, serving as a control for the last group; * $P < 0.05$ compared with sham-operated rats; § $P < 0.05$ compared with respective ARF control.

L-arginine induced, however, a significant increase in the RBF after ARF, in comparison with all other groups with ARF. Moreover, the RBF rose by 27% in the L-Arg + ARF group with respect to the sham-operated group. The RVR was lowered slightly in this group, in comparison with the control groups with ARF and the sham-operated group, and was reduced markedly, compared with the L-NAME + ARF group (Figure 4).

Biochemical parameters are shown in Table 1. No significant differences in plasma sodium concentration were found between the groups. There was an increase in plasma potassium concentration in all groups 24 h after renal ischaemia when compared with the sham-operated group. In the L-NAME + ARF group the increase in plasma concentration of K^+ was significantly higher compared with the relevant 4 weeks-ARF control group. Creatinine clearance decreased in all groups after AFR, but there were no significant differences among the groups. Fractional excretion of Na^+ and K^+ increased 24 h after renal ischaemia in all groups. The highest increase in fractional excretion of sodium was observed in the L-NAME + ARF group. The reabsorption rate of water was reduced in all groups with ARF. However, this reduction was not

statistically significant in the L-Arg+ARF group in comparison with the sham-operated group. The reabsorption rate of Na⁺ was also reduced in all groups 24 h after renal ischaemia and a large decrease was observed after inhibition of NO synthesis.

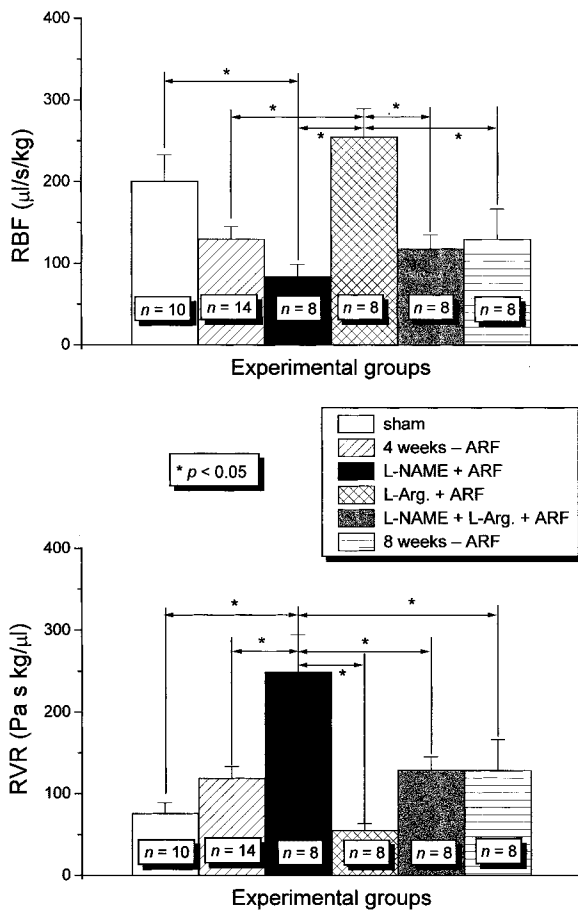


Fig. 4. Renal blood flow (RBF) and renal vascular resistance (RVR) in: sham-operated rats, control rats with acute renal failure (4 weeks-ARF), L-NAME-treated ARF rats, L-arginine treated ARF rats, rats treated first with L-NAME and then with L-Arg (L-NAME + L-Arg + ARF group), and in rats of the (8 weeks-ARF) group, serving as a control for the last group.

Histological examination of the kidney specimens obtained 24 h after ARF revealed that rats treated with L-arginine had minor morphological changes in comparison with all other groups with ARF (Figure 5A). Moreover, there was no significant morphological difference between this group and the sham-operated group. However, the L-NAME+ARF group had significant tubulointerstitial, arteriolar and glomerular lesions. Tubulointerstitial lesions in this group were widespread and intensive and they were characterized by extensive tubular necrosis in the corticomedullary area (Figure 6A). In addition, dilatation of lumina of distal tubuli with huge PAS positive tubular casts was also found to be characteristic of the L-NAME+ARF group. In contrast, in the L-arginine+ARF group only the swelling of proximal tubular cells and different degrees of interstitial oedema were found (Figure 6B). Concerning glomeruli, the L-NAME+ARF group had the most prominent morphological changes. We noticed an increase in both the mesangial matrix and cells in all glomeruli, and in addition, in some glomeruli we revealed mesangial sclerosis or fibrinoid necrosis (Figure 6A). In the L-NAME group without ARF, minor abnormalities of glomeruli were detected, such as a slight increase of the mesangial matrix with or without mesangial cell proliferation. These findings are correlated with those on the morphology of blood vessels. However, the glomeruli of the L-arginine group were almost indistinguishable from those of the sham-operated group. A slight increase of mesangial cell proliferation is rarely observed in this group (Figure 6B). The blood vessels of rats treated with L-NAME after inducing ischaemic ARF showed a wide range of morphological changes; from minor ones such as swelling and proliferation of myointimal cells, to extensive ones such as fibrinoid necrosis (Figure 7A). Before ARF was induced the rats pretreated with L-NAME had some evident morphological abnormalities of the blood vessels, such as vacuolization of myointimal cells, foam transformation of media, or even myoelastofibrosis in two cases. Thus, the blood vessel lesions in L-NAME rats were more pronounced after ARF than before it. In L-arginine-

Table 1. Biochemical parameters in: sham operated rats, control rats with ARF (after 4 and 8 weeks), L-NAME treated rats with ARF, L-arginine-treated rats with ARF and rats with ARF, treated with both L-NAME and L-arginine

	Sham (n=10)	ARF-4 weeks (n=14)	L-NAME+ARF (n=8)	L-Arg+ARF (n=8)	L-NAME+L-Arg+ARF (n=8)	ARF-8 weeks (n=8)
P _{Na+} (mmol/l)	142.0±2.1	144.27±0.61	143.3±1.1	139.9±2.4	136.1±1.2	137.4±1.0
P _{K+} (mmol/l)	3.5±0.1	3.92±0.12	5.01±0.41 ^{1,2}	4.72±0.23 ¹	4.67±0.24 ¹	4.88±0.23 ¹
Urine flow (l/min/kg)	13.8±1.1	14.7±1.8	12.6±2.3	11.2±3.5	15.8±2.6	12.0±3.0
C _{CRE} (µl/s/kg)	44.1±4.5	2.93±0.64 ¹	1.31±0.45 ¹	1.01±0.11 ¹	2.12±0.25 ¹	4.0±1.5 ¹
FE _{Na+} (%)	0.17±0.09	2.91±0.72	7.2±1.7 ^{1,2}	6.4±1.3 ¹	4.36±0.51	1.55±0.54
FE _{K+} (%)	34.4±2.2	275±44 ¹	265±29 ¹	330±21 ¹	230±22 ¹	163±33
Reabsorption rate of H ₂ O (%)	99.48±0.04	86.3±2.2 ¹	87.2±2.2 ¹	92.18±0.83	88.6±1.5 ¹	91.9±2.1
Reabsorption rate of Na ⁺ (%)	99.86±0.05	97.09±0.72	90.2±3.0 ^{1,2}	93.6±1.3 ¹	95.64±0.51	97.2±1.4

Values represent means±SEM; P, plasma; C_{CRE}, creatinine clearance; FE_x, fractional excretion of x; ¹P<0.05 compared with sham-operated rats; ²P<0.05 compared with respective ARF control.

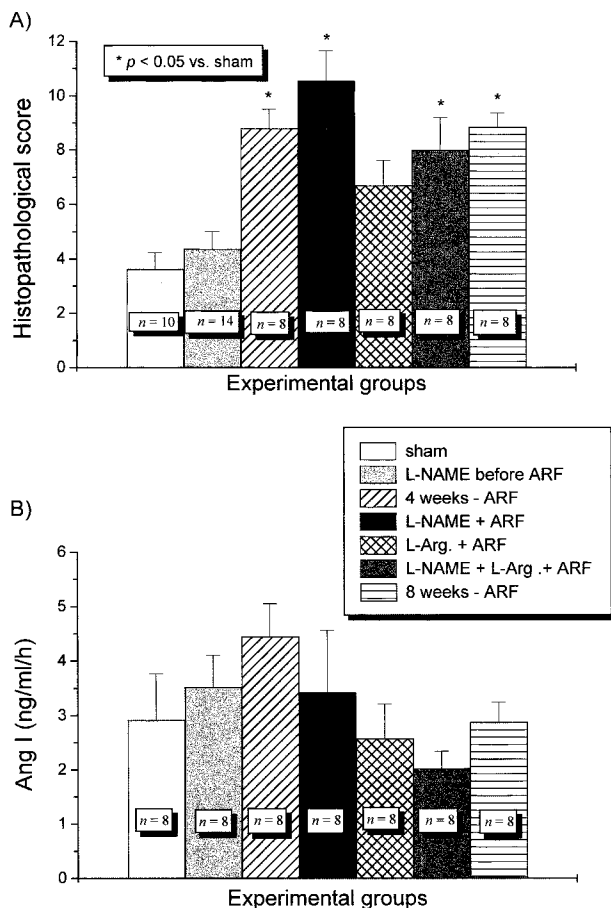


Fig. 5. (A) Histopathological score and (B) Plasma concentration of angiotensin I (ANG I) in: sham-operated rats, control rats with acute renal failure (4 weeks-ARF), L-NAME-treated rats before and after ARF, L-arginine treated ARF rats, rats treated first with L-NAME and then with L-Arg (L-NAME + L-Arg + ARF group), and in rats of the (8 weeks-ARF) group, serving as a control for the last group.

treated rats slight necrotic arteriolar lesions were rarely detected (Figure 7B).

Figure 5B shows that 24 h after ARF the plasma concentration of angiotensin I (ANG I) was slightly but non-significantly higher in the 4 weeks-ARF rats when compared with the sham-operated rats. The treatment with L-NAME was not accompanied by any significant alteration in the plasma concentration of ANG I, while in the group treated with L-arginine, PRA had a tendency to fall in relation to the relevant 4 weeks-ARF control group. As for the L-NAME + L-Arg + ARF group, the circulating level of ANG I was not different when compared with the respective 8 weeks-ARF control animals.

Discussion

To test the view that chronic administration of L-NAME inhibits NO synthesis, we examined the vaso-relaxant effect of Ach (this effect is often used as an indicator of NO synthase activity, for example

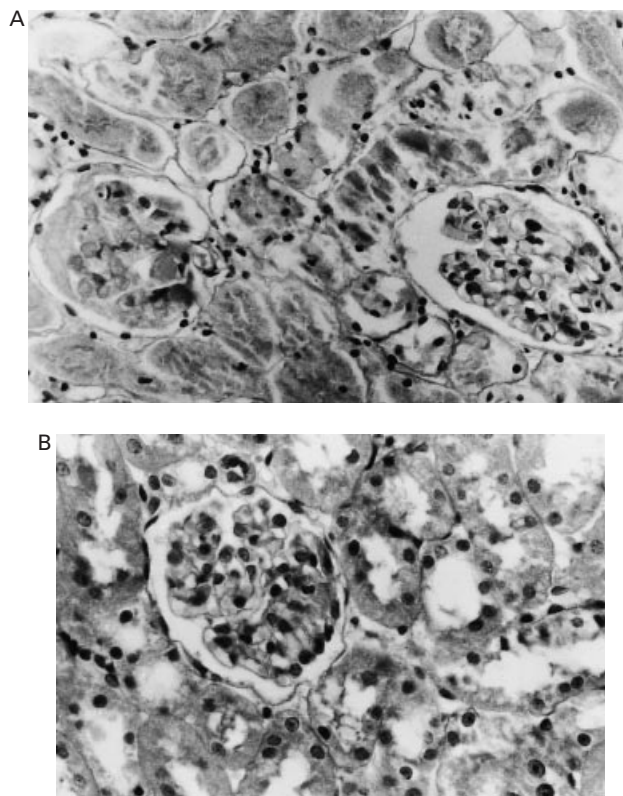


Fig. 6. (A) Necrotic glomerulus and tubuli (left) in comparison to the well preserved glomerulus (right) with slight mesangial cell proliferation in L-NAME treated rats with ARF ($\times 320$); (B) Almost normal glomerulus with slight mesangial cell proliferation and slight increase of mesangial matrix in L-arginine treated rats with ARF; swelling of the proximal tubular cells is also shown ($\times 400$).

Ref. [13]). What we have found is a considerably weaker response to Ach in rats pretreated with L-NAME. This can be taken as a strong indication of the NO synthesis inhibition in L-NAME-pretreated animals. However, even though the response was weak it did not disappear completely because Ach may act on the endothelium in ways that are not NO synthase-dependent, such as by releasing the endothelial-derived hyperpolarizing factor (EDHF).

Our results have shown that long-term stimulation of NO release by L-arginine improves renal haemodynamic parameters in the ischaemic form of ARF, whereas they are worsened by the inhibition of NO synthesis. We also showed that this aggravation provoked by L-NAME can be reversed by L-arginine. These observations parallel those of Rivas-Cabañero *et al.* [13], showing that NO inhibition aggravates gentamicin-induced renal failure in rats, and that of Schramm *et al.* [15] and Schmidt *et al.* [16], demonstrating beneficial effects of NO on the renal function in toxic ARF, produced in rats with uranyl nitrate. Chintala and co-workers [17] found that acute inhibition of endothelial-derived relaxing factor (EDRF) aggravates ischaemic acute renal failure in anesthetized rats. The experimental protocol in their experiments was similar to ours. However, they admin-

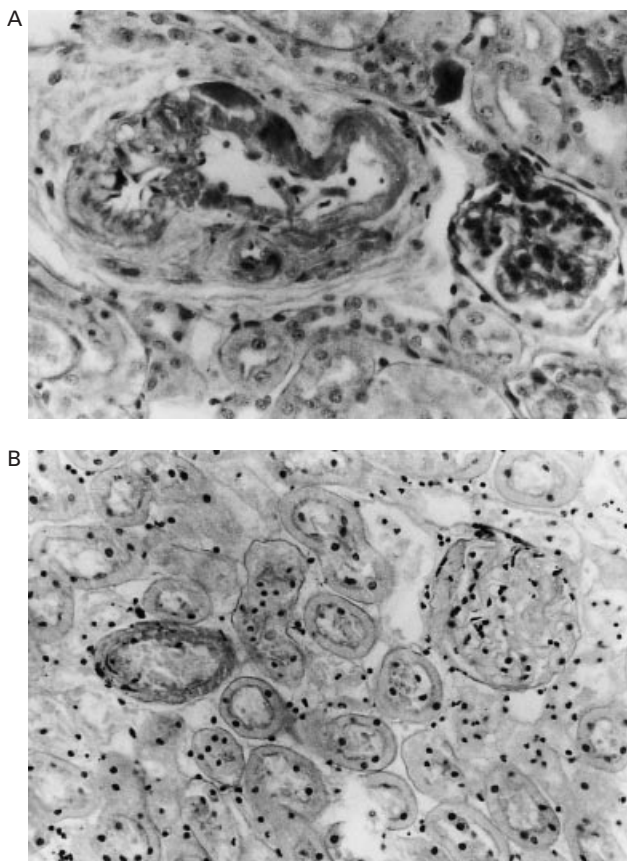


Fig. 7. (A) Segmental fibrinoid necrosis of interlobular artery with collapsed glomerulus in L-NAME treated rats with ARF ($\times 320$); (B) Intensive interstitial oedema with necrosis of the small artery in L-arginine treated rats with ARF ($\times 250$).

istered an NO synthase inhibitor (*N*^G-monomethyl-L-arginine, L-NMMA) or NO precursor (L-arginine), or both of them in an infusion that started 30 min before the occlusion of the left renal artery (lasting 40 min) and continued throughout the experiment (220 min). The authors found that NO synthesis inhibition exacerbated ischaemia-induced changes in renal function, whereas the infusion of L-arginine resulted in improved renal plasma flow (RPF) and glomerular filtration rate (GFR) in the ischaemic kidney.

In our experiments chronic application of L-NAME, 4 weeks before the induction of ARF, resulted in a significant rise in systolic blood pressure, as described previously by Baylis *et al.* [18] and Oliviera *et al.* [19], suggesting that L-NAME was effective in inhibiting NO synthesis.

Induction of ARF did not significantly alter the systemic haemodynamic parameters (SHP). In the ARF group treated with L-NAME, MAP and TVR increased, while CO had a tendency to decrease (Figure 3). However, the same changes of those parameters were observed in the L-NAME-treated rats without ARF (unpublished data). In neither group, i.e. the L-Arg + ARF and the L-NAME + L-Arg + ARF, were the SHP improved. It can be concluded that ARF does not modify the effects of EDRF/NO pathway

inhibition or stimulation on the systemic haemodynamics.

This study has shown, however, that renal haemodynamic alterations induced by ARF, where NO synthesis was inhibited by L-NAME, were worse than those induced by ARF alone. The aggravation of the ischaemia-induced renal vasoconstriction caused by L-NAME suggests that endogenous NO is functionally present and may be an important mediator regulating renal haemodynamic parameters in ARF. Conger *et al.* [20] demonstrated that endothelium-dependent relaxation is clearly impaired after ischaemia. In a recent publication [21] however, these authors concluded that NOS/NO activity is in fact highest above baseline 1-week after norepinephrine-induced ARF and cannot be increased further by exogenous stimuli of NOS activity. Schramm *et al.* [15] suggested that renal ischaemia, which injures endothelial cells, reduced the production of NO in them. On the contrary, Chintala *et al.* [17] summarized that synthesis of EDRF is probably turned on maximally by the ischaemic insult because treatment with L-arginine resulted in only a modest improvement of RPF and GFR in the ischaemic kidney. In our study however, long-term treatment with L-arginine resulted in a marked increase in the renal blood flow 24 h after renal ischaemia. Namely, RBF became increased by 96% in the L-arginine-treated rats with ARF compared with the group with ARF alone (Figure 4). This strongly suggests that release of NO does not reach its maximum 24 h after ischaemia-reperfusion injury.

Creatinine clearance, C_{CRE} , is not significantly different among groups with ARF, irrespective of their treatment, and is lowest in L-arginine-pretreated rats with ARF. This is because C_{CRE} is not a suitable indicator of glomerular filtration in rats treated with L-arginine (and only in them) since creatinine arises as a waste product in the decay of L-arginine. However, it seemed almost inconceivable to add a more appropriate method, e.g. determination of inulin clearance, to our procedure, which was already very difficult for the animals.

From definitions of the reabsorption rates of Na and water, stems the idea that an increase in creatinine clearance increases both of them. This means that they are actually both greater in ARF rats treated with L-arginine than their values calculated by using measured creatinine clearance. Nevertheless, the reabsorption rate of water and Na⁺ was higher in ARF rats treated with L-arginine than in L-NAME-treated rats with ARF (Table 1). Moreover, the reabsorption rate of water in the L-arginine group was not different compared with the sham-operated group. These effects of L-arginine are most likely produced by stimulating reabsorption of water and Na⁺ at the tubular site. This hypothesis was substantiated by histopathological findings in the L-arginine group where only swelling of the proximal tubular cells was observed (Fig. 6B). Along the same lines, Negita and co-workers [22] reported that vacuolization and desquamation of epithelial cells from the renal tubules were slight 24 h

after reperfusion in groups treated with L-arginine or sodium nitroprusside compared with those in the control ARF group and the group treated with L-NAME. In this study, the left renal artery and vein were clamped for 90 min and the animals were treated either with a NO synthesis inhibitor or with two stimulators during various periods before reperfusion.

Previous studies have indicated that there is a growing ambiguity about the role of NO and its metabolic product peroxynitrite (ONOO^-) in the pathophysiology of ARF. ONOO^- is thought to mediate the toxic action of NO and superoxide anion in hypoxia–reperfusion injury of different organs, including the kidney [23]. But the efficiency of peroxynitrite-dependent reactions was found to be highest at equimolar concentrations of O_2^- and NO, which suggested that an excess of either reactant could protect against peroxynitrite *in vivo* [24]. This finding led Bartosz [24] to conclude that the common opinion ('small amounts of NO are beneficial while large amounts of NO are toxic') should be revised since higher doses of NO may suppress peroxynitrite reactions. Xia *et al.* [25] experimented with NOS-transfected human renal tubular epithelial cells and found that elimination of L-arginine from the medium resulted in ONOO^- production and cell injury. However, some experiments *in vitro* showed that two NO synthesis inhibitors, L-NAME and L-NMMA, protected renal tubular epithelium against hypoxic injury. Nevertheless, the results obtained in experiments *in vivo* showed the opposite. Namely, administration of nonselective NO synthesis inhibitors further compromised renal function in various models of ARF *in vivo* [13,17,22]. Noiri *et al.* [26] reasoned that the existing uncertainty about the role of NO in ARF is in part due to a lack of selective NOS inhibitors. These authors found that *in vivo* targeting of inducible NOS (iNOS) with oligodeoxynucleotides protects rat kidney during ischaemia. When the function of all isoforms of NOS is blocked (as with non-selective inhibitor), the authors concluded, the deleterious consequences of inhibiting endothelial constitutive NOS invariably prevail over the possible benefits of inhibiting iNOS. Taken together, these findings, imply that the degree of injury is linked to the iNOS, while restoration of renal function after noxious stimuli depends on constitutive NOS.

The present communication is the first report about post-ischaemic ARF after a long-term NO release stimulation or inhibition. Our main finding is that, besides the improvement of renal haemodynamic parameters, treatment with L-arginine reduces tubular cell injury in acute post-ischaemic renal failure. This is most clearly seen in observing tubular epithelial cell morphology in rats treated with L-arginine: the changes found were subtle (Figure 6B).

However, we found most prominent lesions in the L-NAME + ARF group: large areas of corticomedullar necrosis and heavy damage of the tubular epithelial cells with a lot of intratubular casts. But, this aggravation of changes in renal morphology can be reversed by L-arginine.

To conclude, acute post-ischaemic renal failure may be alleviated by administering the NO substrate (L-arginine) and NO acts cytoprotectively in ischaemia–reperfusion injury of rat kidneys.

The improvement in the course of ARF due to the administration of L-arginine observed in this study implies that the administration of the NO synthesis donor to patients with ARF would yield a favourable effect.

However, all parameters of the ARF are obviously worse in rats with chronic inhibition of the NO synthesis. This indicates that a considerably poorer course of ARF can be expected in all diseases in which either synthesis and/or action of NO is decreased chronically. Further support for this conclusion comes from morphological changes, found in kidneys, in the group treated with L-NAME before induction of ARF: minor abnormalities of glomeruli, vacuolization of myointimal cells and foam transformation of blood vessel media. When ARF is induced in the group pre-treated with L-NAME, however, morphological changes in all renal structures are much stronger than in the group with ARF only. This also may contribute to the understanding of the high mortality in ARF.

Numerous authors who investigated mechanisms of NO activity have shown [27,28] that NO plays an important role in the regulation of endocrine functions (e.g. control of the secretion of pancreatic, hypothalamic, pituitary and other hormones). It was shown that NO inhibits the synthesis of endothelin [29]. Nevertheless, our previous experiments suggested that NO is not involved in endothelin release caused by ARF [30]. Evidence is also emerging that NO contributes to the regulation of the secretion of renin, which was among the topics of our investigation.

The data obtained in our study show that plasma concentration of ANG I was increased non-significantly 24 h after ARF. The plasma renin activity has been repeatedly shown to be increased in the initial stages of both clinical and experimental forms of ARF [5,7]. Although these observations are consistent with the possibility that the renin–angiotensin system (RAS) is involved in ARF, they do not establish a causal connection between the activation of the RAS and the subsequent development of ARF. Furthermore, it has been shown that the PRA may return to the control level although ARF still continuous or persists [31].

The effects of NO on renin release and intrarenal formation of ANG II remain controversial [32]. Reid and Chiu [33] quoted some studies, their results suggesting a stimulatory role for the L-arginine–NO pathway in the control of renin secretion. However, under different circumstances (e.g. after long-term treatment) blockade of NO synthesis increases renin secretion. The development of hypertension in Sprague–Dawley rats, produced by chronic treatment with L-NAME depends on intactness of RAS, but L-NAME can clearly increase blood pressure independent of any involvement of ANG II, concluded Melaragno *et al.* [34]. An apparent inconsistency in the above-mentioned reports may be resolved if a dual effect of

NO on renin release is assumed. After an initial transient inhibitory effect, there appears to be a delayed but sustained stimulatory effect on renin formation [35]. Schriker *et al.* [36] observed that the inhibitors of NO formation attenuate plasma renin activity and also reduce renin mRNA levels in the stenosed kidney of two-kidney one-clip Goldblatt hypertensive rats, suggesting that NO plays the role of an activator of renin gene expression. Guan *et al.* [37] demonstrated that L-arginine–NO and RAS were interactive at various levels. Nitric oxide might subserve mechanisms that control renin release, but the precise nature of the regulatory influence, inhibitory or stimulatory, has been the subject of conflicting reports [38]. Nevertheless, some authors have indicated that NO is a physiological antagonist of renin and that NO synthesis inhibitors enhance renin release [39,40]. On the one hand, Higashi *et al.* [2] found that activity of the angiotensin-converting enzyme (ACE) was inhibited by intravenous administration of L-arginine in humans and that the level of ANG II was decreased in parallel with changes in ACE activity. On the other hand, their findings showed that L-arginine infusion did not alter PRA in normal subjects.

Our results showed that, 24 h after ARF in the group treated with L-arginine, PRA had a tendency to decrease. This may contribute to improvements in renal haemodynamics in the L-arginine-treated group with ARF. However, treatment with L-NAME did not change PRA. Results obtained in the L-NAME-treated groups with and without ARF could be explained by two opposing mechanisms that appear to affect PRA. The increased blood pressure may tend to reduce renin release [41], whereas inhibition of NO synthesis may tend to enhance renin release [42]. Therefore, we can assume that under the experimental conditions used in this study there exists an equilibrium between both mechanisms. PRA remained unchanged in rats treated with both L-NAME and L-arginine, compared with their respective control animals, because all haemodynamic parameters gradually returned to their respective control levels.

In summary, our results have shown that long-term stimulation of NO release by L-arginine improves renal haemodynamic parameters in ischaemic form of ARF, whereas inhibition of NO synthesis worsens them. This aggravation (provoked by L-NAME) can be reversed by L-arginine. Treatment with L-arginine reduces tubular cell injury in acute post-ischaemic renal failure. This is most clearly seen in observing tubular epithelial cell morphology in rats treated with L-arginine: the changes found were subtle. We conclude that NO acts cytoprotectively in ischaemia–reperfusion injury in rat kidney. An improvement of the course of the ARF due to administration of L-arginine observed in this study implies that the administration of the NO synthesis donor to patients with ischaemic ARF would yield a favourable effect. Plasma renin activity had a tendency to decrease in rats treated with L-arginine. This may contribute to an improvement of renal

haemodynamics in the L-arginine treated group with ARF.

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