British Journal of Pharmacology (1996) 117, 1348–1354

Calcium entry and 5-HT_2 receptor blockade in oliguric ischaemic acute renal failure: effects of levemopamil in conscious rats

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1 Unilateral left renal artery occlusion for 1 h in a group of 8 untreated female Sprague-Dawley rats resulted in oliguric acute renal failure (ARF) persisting for more than 6 h after reflow, i.e. after reperfusion of the kidney by removal of the arterial clamp. In a second group of 8 rats with left unilateral ARF the effects of levemopamil (L), a calcium entry blocker with 5-hydroxytryptamine$_2$ (5-HT_2) receptor antagonistic properties, were studied. Rats received L as a continuous infusion (6 mg kg^{-1} h^{-1}) from 1 h before ischaemia until 6 h after reflow.

2 Endogenous creatinine clearance, an estimate of glomerular filtration rate (GFR), of left ischaemic kidneys of untreated rats was almost completely abolished and urine flow was 0.05 ± 0.02 and 0.03 ± 0.01 ml h^{-1} 100 g^{-1} body weight (body wt.) at 2 and at 6 h of reflow, respectively. In contrast, left ischaemic kidneys of L-treated rats revealed significantly higher GFR (0.10 ± 0.02 and 0.03 ± 0.01 ml min^{-1} g^{-1} kidney weight (k.wt.); $P < 0.01$) and urine flow (0.51 ± 0.05 and 0.15 ± 0.04 ml h^{-1} 100 g^{-1} body wt.; $P < 0.05$) at 2 and 6 h of reflow, respectively.

3 At 6 h of reflow, mitochondria from the cortex of left ischaemic kidneys of untreated rats showed significantly reduced ATP synthesis when compared to right intact kidneys (0.06 ± 0.02 vs 0.26 ± 0.02 μmol ATP mg^{-1} protein min^{-1} ($P < 0.01$)). In contrast, in L-treated rats, ATP synthesis of left ischaemic kidneys was largely preserved (0.17 ± 0.01 μmol ATP mg^{-1} protein min^{-1}).

4 Ischaemia of left kidneys resulted in a significant decrease in medullary Na-K-ATPase activity to 9.6 ± 2.4 as compared to 20.4 ± 3.7 μmol P_i h^{-1} mg^{-1} protein in the intact right kidneys which was not prevented by L (9.4 ± 2.4 μmol P_i h^{-1} mg^{-1} protein).

5 In untreated rats the calcium content in cortical mitochondria from left ischaemic kidneys had risen 2 fold to 23.0 ± 1.8 at 6 h of reflow as compared to 12.2 ± 0.3 nmol mg^{-1} protein in right intact kidneys ($P < 0.01$). This rise in mitochondrial calcium was not significantly attenuated by treatment with L (19.9 ± 1.7 nmol mg^{-1} protein).

6 The results show that L transiently converted oliguria into non-oliguria during the early phase after reflow in ischaemic ARF, i.e. after reperfusion following 1 h of complete interruption of renal perfusion. The present data suggest indirectly that the 5-HT_2-antagonistic properties of L rather than its calcium channel blocking action maintains GFR at low level and protects mitochondrial function early after reflow in this model of ischaemic ARF.

Keywords: Ischaemic acute renal failure; conscious rats; calcium entry blockade; 5-HT receptor blockade; levemopamil

Introduction

Acute renal failure (ARF) is a sudden loss of renal function which is characterized by disturbances of water, electrolyte, and acid-base balance and the retention of metabolic waste products. Despite the impressive progress in intensive care medicine, there is still a high incidence of mortality in patients with ARF, especially in those with oliguric ARF and those with further complicating diseases (Maher & Schreiner, 1962; Kleinknecht *et al.*, 1971; Stott *et al.*, 1972; Minuth *et al.*, 1976). Until recently, more than 70% of ARF in man was due to circulatory failure with renal hypoxia which often resulted in oliguric ARF. Compromised renal and glomerular perfusion with reduced capillary pressure and permeability, loss of renal autoregulation, vascular congestion in the outer medulla, tubular leakage and cast formation have been incriminated as pathogenetic factors (Finn & Chevalier, 1979; Oken, 1981; Smolens & Stein, 1981; Mason *et al.*, 1987; Matthys *et al.*, 1983).

The early phase of ischaemic ARF is characterized by a profound reduction of renal blood flow (RBF) and a loss of autoregulation which result in decreased GFR and reduced oxygen delivery to the tubular epithelium. Tissue damage occurs mainly during subsequent reperfusion (reflow). Early protective measures would therefore be highly desirable and a number of pharmacological interventions were employed in the past in experimental and clinical studies with respect to the various pathophysiological mechanisms which include intrarenal haemodynamic alterations and cell damage by calcium overload (for review see Mohaupt & Kramer, 1989/1990).

That 5-hydroxytryptamine (5-HT) plays a role in the regulation of renal haemodynamics via 5-HT_2 receptors has been demonstrated by various authors (Lameire *et al.*, 1990; Endlich *et al.*, 1993). The potential role of 5-HT in acute renal damage has been studied in the rat kidney after transplantation following warm and cold ischaemia (Mills *et al.*, 1987) as well as following cyclosporine administration (Darlametsos *et al.*, 1995). A pathogenic role of 5-HT was suggested in patients with haemorrhagic fever and ARF (Sidel'nikov & Sivoraksha, 1990). These observations point to a potentially beneficial effect of 5-HT antagonists in acute renal damage.

In the present study we therefore investigated the effects of levemopamil (L) on renal functional and metabolic alterations in conscious rats during the early phase after reflow in acute oliguric ischaemic ARF. Levemopamil, a calcium entry blocker of the phenylalkylamine type, also possesses 5-HT_2-receptor antagonistic properties (Defeudis, 1989) and this drug was found to have a greater potential for tissue protection after

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complete ischaemia than its parent compound, the calcium channel blocker, verapamil (Defeudis, 1989). The additional 5-HT_2 antagonism of L may therefore have distinct therapeutic implications in preventing or treating ARF in the early phase after an hypoxic insult.

Methods

Female, adult Sprague-Dawley rats, weighing 191 to 239 g with free access to water and standard rat chow diet (Altromin) were studied. They were divided into two groups, each of 8 rats, i.e., an untreated group and a levemopamil (L)-treated group. The experiments were performed according to the Declaration of Helsinki with permission No. 26.203.2 BN22, 36/88 from March 03, 1989, of the Federal Animal Protection Committee, Cologne, Nordrhein-Westfalen, Germany.

Under methohexitone anaesthesia (Brevimytal-Na, Lilly GmbH, Giessen, Germany, 2 mg kg^{-1} body weight (body wt.) i.p. and subsequently 0.2 mg kg^{-1} body wt., i.v. when necessary) catheters were placed in the right jugular vein (PE-50), and in the urinary bladder (PE-90). The volume of the bladder was reduced by a suture above the entrance of the right ureter. A PE-10 catheter of 1 cm length was placed into the left ureter which was connected to a PE-50 and PE-90 catheter. Animals were then placed in individual restraining plastic cages which were mounted on a scale. Body weight was monitored throughout the experiments. During the 2nd h after completion of surgery, urine was collected from the left kidney via the ureteral catheter and from the right kidney via the urinary bladder catheter to obtain control data of renal function. At the end of this urine collection period, during short methohexitone anaesthesia, the left renal artery, which was previously separated from surrounding tissue and from the renal vein, was then covered by a strip of parafilm and was completely occluded with a metal clamp. In conscious animals renal artery occlusion was maintained for 1 h and was then released, again under short methohexitone anaesthesia. Urine samples were collected hourly from before ischaemia and during the 6 h of reflow. From 2 h before to 6 h after ischaemia the animals were infused i.v. with 0.45% NaCl/2.5% glucose solution at an average infusion rate of 2 ml h^{-1} to serve as vehicle and to maintain body weight. In the treated group, L was administered i.v. at a rate of 6 mg h^{-1} kg^{-1} body wt. from 1 h before ischaemia until 6 h after reflow. Venous blood (0.5 ml) was collected at 1 h before and 2 h after the ischaemic period. At the end of the experiments, at 6 h of reflow, both kidneys were removed under methohexitone anaesthesia. The animals were killed by aortic puncture for arterial blood sampling.

Kidneys were placed on ice-cold glass plates. Kidney weights after removal of fat and connective tissue of left ischaemic kidneys were similar in untreated and L-treated rats (1.07 ± 0.16 and 1.06 ± 0.16 g, respectively). They were slightly but not significantly greater than those of right intact kidneys from untreated and L-treated rats (0.88 ± 0.11 and 0.87 ± 0.10 g, respectively). Kidneys were then dissected to obtain tissue from cortex, medulla, and papilla. Homogenates of cortex, medulla, and papilla were prepared at a 1:10 (w/v) dilution in a Tenbroek glass homogenizer at 4°C with a buffer solution containing 0.25 mol l^{-1} sucrose, 38 mmol l^{-1} Tris-HCl, pH 6.8 for determination of Na-K-ATPase activities. Cortical and papillary homogenates were further diluted 1:10 (v/v) and medullary homogenates 1:20 (v/v) with the same buffer solution.

Total ATPase activity was measured in incubation tubes containing 0.8 ml of substrate solution providing final concentrations of (mmol l^{-1}): ATP 3, MgCl 3, NaCl 100, KCl 20 and imidazole-HCl 100, pH 7.2, placed in a water bath at 37°C. The reaction was started by adding 0.2 ml of enzyme preparation and was stopped after 10 min by adding 1 ml of ice-cold 10% trichloroacetic acid (TCA). After centrifugation at 1,700 *g* for 5 min, 1.0 ml of the supernatant was assayed for inorganic phosphate (P_i) according to the method of Taussky & Shorr (1953). Mg^{2+}-ATPase activity was assayed in the presence of 10^{-3}M ouabain. Protein determination was carried out by the method of Lowry *et al.* (1951). Na-K-ATPase was calculated as the difference between total ATPase and Mg^{2+}-ATPase activities and expressed as μmol P_i liberated h^{-1} mg^{-1} protein. All determinations were performed in triplicate. The normal values for Na-K-ATPase activities in tissue homogenates of renal cortex, medulla and papilla from 5 female Sprague-Dawley rats at 6 h after sham-operation were 7.0 ± 0.9, 13.8 ± 2.2 and 3.7 ± 0.7 μmol P_i h^{-1} mg^{-1} protein, respectively.

For isolation of renal mitochondria, homogenates of cortical tissue were also obtained at the end of the experiments, i.e. at 6 h of reflow, and were prepared at 4°C with a solution of 0.25 mol l^{-1} sucrose, 2 mmol l^{-1} EDTA, 10 mmol l^{-1} Tris-HCl, pH 7.4. Homogenates were then centrifuged at 500 *g* for 10 min at 2°C. The supernatant was centrifuged at 6,000 *g* for 7 min at 4°C. The 6,000 *g* pellet was washed twice and resuspended in the sucrose-Tris-EDTA-solution and 0.2 ml of the suspension retained for protein determinations. Mitochondrial function was measured according to Estabrook (1976) and Weinbach (1961) with slight modification using a Clark oxymeter (Yellow Springs Instruments, Colorado Springs, U.S.A.). To a reaction solution (2.7 ml) containing 0.225 mol l^{-1} sucrose, 2 mmol l^{-1} KH_2PO_4, 0.5 mmol l^{-1} EDTA, 20 mmol l^{-1} KCl, 20 mmol l^{-1} Tris, and 3.3 mmol l^{-1} succinate at pH 7.4 and a temperature of 25°C, which was equilibrated with room air, 5 mg of lyophilized bovine serum albumin (BSA) was added (Toader *et al.*, 1976). The reaction was started by the addition of 0.1 ml of mitochondrial suspension into the closed reaction chamber. After a constant 'state 4-respiration' (baseline oxygen consumption by intact mitochondria in the presence of excess substrate, e.g. succinate, but in the absence of stimuli, e.g. ADP) was obtained, 0.3 μmol ADP dissolved in 0.1 ml of distilled water was injected into the reaction chamber ('state 3-respiration', i.e. maximum speed of electron transport of the respiratory chain in the presence of ADP). After constant 'phase 4-respiration' was again reached, addition of ADP was repeated. The change

Table 1 Baseline values for urine volume (V) and osmolarity (U_{osm}), urine sodium concentration (U_{Na}) and sodium clearance (CL_{Na}), and fractional sodium excretion (FE_{Na}) from left and right kidneys before left renal artery occlusion in untreated ($n=8$) and levemopamil (L)-treated ($n=8$) conscious rats

	Untreated rats		*L-treated rats*	
	left (pre-ischaemic)	*right (intact)*	*left (pre-ischaemic)*	*right (intact)*
V (ml h^{-1} 100 g^{-1})	0.78 ± 0.45	0.70 ± 0.40	0.72 ± 0.26	0.70 ± 0.56
U_{osm} (mosm l^{-1})	402 ± 140	525 ± 225	403 ± 167	415 ± 183
U_{Na} (mmol l^{-1})	13.2 ± 5.3	18.7 ± 10.6	22.6 ± 13.5	22.2 ± 16.4
CL_{Na} (μl min^{-1} 100 g^{-1} body wt.)	1.2 ± 0.8	1.5 ± 1.1	1.9 ± 0.8	1.8 ± 1.1
FE_{Na} (%)	0.4 ± 0.3	0.5 ± 0.3	0.7 ± 0.3	0.6 ± 0.5

in oxygen content was recorded with an automatic recorder. The respiratory control ratio (RCR, i.e. the ratio of oxygen consumption in the presence divided by that in the absence of ADP (state 3/state 4)), a marker of the functional integrity of isolated mitochondria, the ADP/O-ratio, i.e. the number of inorganic phosphates used per oxygen atom to form ATP, and the rate of ATP synthesis were calculated according to conventional formulae.

Mitochondrial calcium concentration was determined by atomic absorption spectrophotometry after treating mitochondrial suspensions with concentrated HNO_3 and was expressed as nmol Ca mg^{-1} protein.

Concentrations of sodium and potassium in serum and urine were measured by flame photometry. Creatinine concentrations in serum and urine were determined by the JAFFE-reaction (with picrinic acid) after adsorption to Fuller's earth for removal of chromogens present in rat serum. The clearance (CL) of endogenous creatinine was calculated from urinary creatinine excretion per min divided by serum creatinine concentration. Osmolarities in serum and urine samples were determined by freezing point depression in a Knauer semiautomatic microosmometer. All determinations were performed in duplicate.

Statistical analysis

Data were analysed with Student's two-tailed *t* test after testing for standard normal distribution or, when appropriate, with Wilcoxon's signed rank test. *P* values <0.05 were considered statistically significant. If not otherwise stated, results are presented as means $\pm$ s.d.

Results

Functional parameters

During the control period before ischaemia no significant differences in urinary volume, osmolarity, and sodium concentration or in the clearance and fractional excretion of sodium were noted between left and right kidneys from untreated or L-treated rats (Table 1).

In L-treated rats urine flow rates from left ischaemic kidneys were significantly ($P<0.05$) higher after the release of the arterial clamp than those of untreated rats (0.52 ± 0.21 vs 0.06 ± 0.05 ml h^{-1} 100 g^{-1} body wt. at 1 h of reflow) and remained higher up to 6 h of reflow (0.15 ± 0.11 vs 0.03 ± 0.02 ml h^{-1} 100 g^{-1} body wt.; $P<0.05$) (Figure 1). Urine flow from right intact kidneys rose progressively during the 6 h of reflow to 2.16 ± 0.37 in untreated and to 1.75 ± 0.40 ml h^{-1} 100 g^{-1} body wt. in L-treated rats (Figure 1).

Osmolarities and sodium concentrations of urines from left ischaemic kidneys from both untreated and L-treated rats were close to plasma tonicity and concentration, respectively, throughout the period of reflow (Figure 2).

Endogenous creatinine clearances of untreated and L-treated rats were similar before ischaemia (Table 2). Creatinine clearance of left ischaemic kidneys from untreated animals was not measurable at 2 and 6 h of reflow because of lack of urine. In contrast, creatinine clearance was maintained in L-treated rats at 2 and 6 h of reflow at approximately 13% and 4% of pre-ischaemic values, respectively. Creatinine clearance of the right intact kidney was slightly but significantly lower ($P<0.05$) in L-treated than in untreated rats (Table 2).

Metabolic parameters

Ischaemia of the left kidney resulted in a significant decrease in medullary Na-K-ATPase activity when compared to the intact right kidney. This decrease in medullary enzyme activity of the ischaemic left kidney was also noted in L-treated rats (Table 3). Moreover, treatment with L was associated with a small but significant suppression of medullary ($P<0.01$) and papillary Na-K-ATPase activities ($P<0.05$) of the right intact kidneys (Table 3).

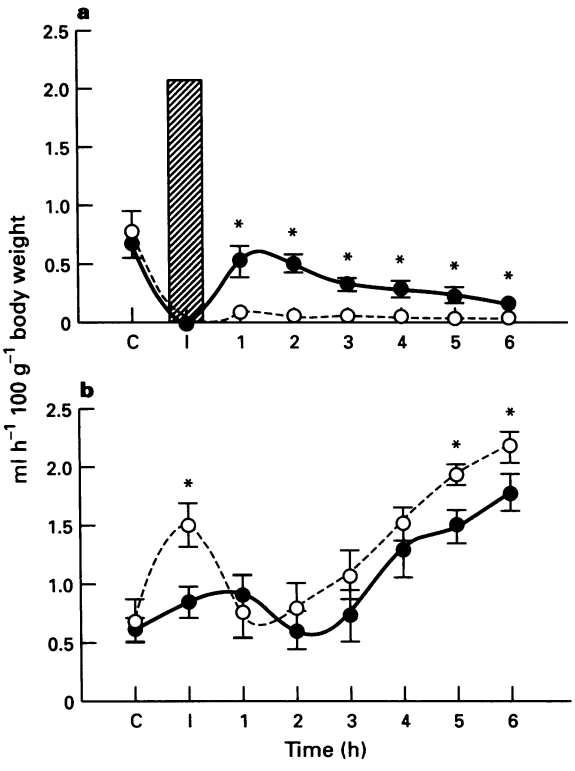

Figure 1 Urine volume of left ischemic (a) and right intact kidneys (b) before (C), during (I), and after 1 h of unilateral occlusion (ischaemia = I) of the left renal artery from untreated (dotted lines) and levemopamil (L)-treated (solid lines) animals (means $\pm$ s.e.mean); $*P<0.05$ for L-treated vs untreated animals.

At 6 h of reflow the respiratory control ratios (RCR) of renal cortical mitochondria from left and right kidneys were similar in untreated and L-treated rats (Table 3). In left ischaemic kidneys of untreated rats the ADP/O-ratio of cortical mitochondria was significantly lower than in right intact kidneys ($P<0.05$). In contrast, in L-treated rats the ADP/O-ratio of cortical mitochondria from left ischaemic kidneys was normal and similar to that of right intact kidneys (Table 3).

The calculated rate of mitochondrial ATP synthesis was thus significantly lower in left ischaemic than in right intact kidneys of untreated rats ($P<0.01$). In contrast, it was significantly ($P<0.05$) higher in ischaemic left kidneys of L-treated rats ($P<0.05$) but did not reach normal values of right intact kidneys (Table 3).

The calcium concentration in cortical mitochondria of left ischaemic kidneys of untreated rats was significantly higher than that of right intact kidneys (23.0 ± 4.8 vs 12.2 ± 0.8 nmol mg^{-1} protein; $P<0.01$). Similarly, in L-treated rats mitochondrial calcium concentration of left ischaemic kidneys was also higher than that of right intact kidneys (19.9 ± 4.5 vs 9.1 ± 1.6 nmol mg^{-1} protein; $P<0.01$) (Figure 3).

Discussion

Hypoxic injury to the kidney results in cell damage due to inadequate energy provision (Gronow & Cohen, 1984). More recent findings in experimental ARF show that functional and metabolic sequelae of hypoxic injury to the kidney include vascular congestion in the inner stripe of the outer medulla (Mason *et al.*, 1987) and impaired oxygen supply to the renal cortex (Schurek, 1990). Thus, despite rapid normalization of

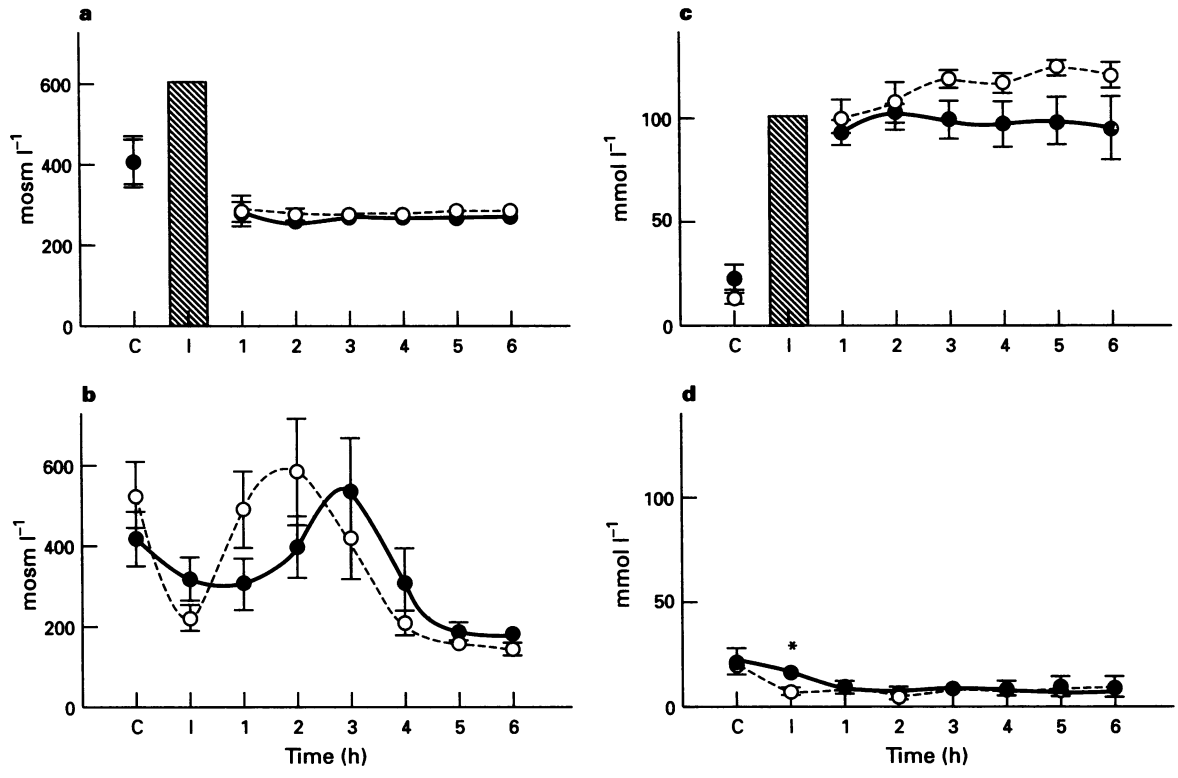

Figure 2 Urine osmolarity (a,b) and sodium concentration (c,d) of left ischaemic (a,c) and right intact kidneys (b,d) before (C), during (I), and after 1 h of unilateral occlusion (ischaemia = I) of the left renal artery from untreated (dotted lines) and levemopamil (L)-treated animals (solid lines), (means ± s.e.mean); $*P < 0.05$ for L-treated vs untreated rats.

Table 2 Glomerular filtration rate (GFR) of left and right kidneys before and after 1 h of complete occlusion of the renal artery from untreated ($n = 8$) and levemopamil (L)-treated ($n = 8$) conscious rats

	Glomerular filtration rate (GFR) (ml min^{-1} g^{-1} k. wt.)			
	Untreated rats		*L-treated rats*	
	left (ischaemic)	*right (intact)*	*left (ischaemic)*	*right (intact)*
Pre-ischaemia	0.77 ± 0.27	0.78 ± 0.32	0.76 ± 0.21	0.87 ± 0.42
2 h of reflow	ND[$]	0.76 ± 0.19	0.10 ± 0.05*	0.60 ± 0.41
6 h of reflow	ND[$]	0.92 ± 0.24	0.03 ± 0.02*	0.73 ± 0.21†

* $P < 0.05$ left ischaemic kidneys of L-treated vs untreated rats.
† $P < 0.05$ right intact kidneys of L-treated vs untreated rats.
$ ND: not detectable because of lack of urine.

Table 3 Na-K-ATPase activities in renal cortex, medulla and papilla, and respiratory control ratio (RCR), ADP/O ratio and rate of ATP synthesis of cortical mitochondria from left and right kidneys of untreated and L-treated rats at 6 h of reflow after 1 h of complete occlusion of the left renal artery

	Untreated rats		*L-treated rats*	
	left (ischaemic)	*right (intact)*	*left (ischaemic)*	*right (intact)*
Na-K-ATPase activities (μmol P_i h^{-1} mg^{-1} protein)				
cortex	7.0 ± 2.9	6.8 ± 1.9	6.9 ± 2.1	4.8 ± 1.1
medulla	9.6 ± 2.4**	20.4 ± 3.7†	9.4 ± 1.6	13.4 ± 2.6
papilla	5.3 ± 2.6	7.0 ± 2.6	3.5 ± 0.8	4.8 ± 1.1
Respiratory control ratio (RCR)	2.0 ± 0.8	2.8 ± 0.8	2.3 ± 0.5	2.3 ± 0.3
ADP/O ratio	1.3 ± 0.8*	2.2 ± 0.2	2.4 ± 0.3	2.3 ± 0.5
ATP synthesis (μmol min^{-1} mg^{-1} protein)	0.06 ± 0.05**	0.26 ± 0.05	0.17 ± 0.03††	0.25 ± 0.03

* $P < 00.05$ and ** $P < 0.01$ for left ischaemic vs right intact kidneys in untreated rats.
† $P < 0.05$ right intact kidneys of untreated vs L-treated rats.
†† $P < 0.01$ left ischaemic kidneys of L-treated vs untreated rats.

total renal blood flow after reperfusion (reflow), medullary blood flow remains markedly reduced (Karlberg *et al.*, 1983) and probably results from hypoxic swelling of endothelial and interstitial cells leading to compression of the confluent vessels in the outer medullary strip which drain the blood from the inner strip and deep medullary tissue (Mason *et al.*, 1987; Bayati *et al.*, 1990). Consequently, oxygen supply is further reduced in an area which normally functions at the verge of hypoxia (Epstein *et al.*, 1982; Rosen & Brezis, 1988). In addition, the renal cortex, which normally exhibits a high blood flow, may also suffer from compromised oxygen supply. Thus, by comparing regional cortical with systemic arterial oxygen saturation, it has been shown that enhanced arterio-venous oxygen diffusion, i.e. shunting in the preglomerular vasculature, partly prevents oxygen from reaching the outer cortex (Schurek *et al.*, 1990). Therefore, cortical tissue, which has only a small capacity for anaerobic glycolysis, is at greater risk of hypoxia than hitherto assumed.

New insights into the pathophysiological events of ischaemic ARF suggest that intracellular calcium may play an important role in cellular damage (Cheung *et al.*, 1986; Schrier *et al.*, 1987; Weinberg, 1991). This is supported by experimental (Burcke *et al.*, 1984; Diepenink *et al.*, 1986; Russell & Churchill, 1989; Schrier, 1991) and clinical (Neumayer & Wagner, 1987; Neumayer *et al.*, 1989) demonstrations of the protective or preventive potential of calcium channel blockers in various forms of ARF and in transplanted kidneys.

More recently, 5-HT was demonstrated to be a potent vasoconstrictor in the kidney (Endlich *et al.*, 1993) and also to play a pathogenetic role in acute clinical (Sidel'nikov & Sivoraksha, 1990) and experimental renal damage (Darlametsos *et al.*, 1995). 5-HT is released from enterochromaffin cells and, more importantly, from platelets, but may also be produced in proximal tubule cells (Sole *et al.*, 1986). Its renal haemodynamic effects can be attributed to a direct action on renal (Blackshear *et al.*, 1986) and specifically renal vascular (Endlich *et al.*, 1993) 5-HT receptors. However, equivocal results have been obtained with respect to the directional changes in vascular tone and on their mediation through 5-HT_1- or 5-HT_2-receptors (Dabire *et al.*, 1990). This may be related to the multiple intracellular signalling pathways, e.g. mediating contraction and long-term functional changes by 5-HT in cultured rat glomerular mesangial cells (Mené *et al.*, 1991). In the kidney these mechanisms also include stimulation of calcium-dependent synthesis of prostanoids (Blackshear *et al.*, 1986; Knauss & Abboud, 1986; Mené *et al.*, 1991) and renin release (Takahashi 1991).

In dog (Takahashi *et al.*, 1992) and rat (Ding *et al.*, 1989) the vasoconstrictor action of 5-HT appears to be mediated by 5-HT_2-receptors (Blackshear *et al.*, 1986), since 5-HT_{1A}-receptors were demonstrated only in the basolateral membrane of the thick ascending limb and of more distal tubules in the cortex and outer medullar but not in blood vessels, glomeruli or inner medulla (Raymond *et al.*, 1993).

In the present study we chose a model of circulatory ARF resulting in oligo-anuria. Thus, 1 h of warm ischaemia of the left kidney was applied in conscious rats to avoid modulatory influences of general anaesthesia and the additional influences of uraemia. In this model we investigated a potential protection by levemopamil (L), a calcium channel blocker with 5-HT (5-HT_2) receptor antagonist activity (Hofmann *et al.*, 1989). This drug has been shown previously in rats to preserve the function of the transplanted kidney following warm and cold ischaemia (Mills *et al.*, 1987) or to protect the kidney from cyclosporine-induced damage (Darlametsos *et al.*, 1995). It also reduced infarct size following cerebral artery occlusion (Nakayama *et al.*, 1988).

Our present results demonstrate that this model of ARF results in nearly complete anuria within 2 h after reflow and loss of glomerular function as reflected by an undetectable endogenous creatinine clearance. Urinary osmolarity and sodium concentration approached plasma isotonicity reflecting severe tubular damage after the ischaemic insult.

L protected glomerular filtration to a certain degree, since creatinine clearance, although at low level, was still measurable at 6 h of reflow. L also maintained urine flow and thus converted oliguria into non-oliguria during the early phase of reflow, but no improvement of tubular function was noted. This agrees with the observation that the severe decrease in medullary Na-K-ATPase activity of left ischaemic kidneys was not prevented by L.

The higher medullary Na-K-ATPase activity of intact right kidneys from untreated rats as compared to that from L-treated rats may be explained by the fact that in untreated rats with an apparently complete loss of glomerular filtration rate

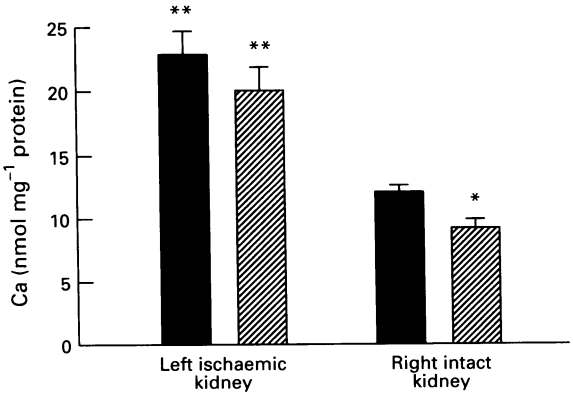

Figure 3 Calcium concentrations in cortical mitochondria of left ischaemic and right intact kidneys at 6 h of reflow from untreated (solid columns) and levemopamil (L)-treated (hatched columns) rats, (means ± s.e.mean); *$P < 0.05$ for right intact kidneys of L-treated vs untreated rats; **$P < 0.01$ for left ischaemic vs right intact kidneys of untreated and L-treated rats.

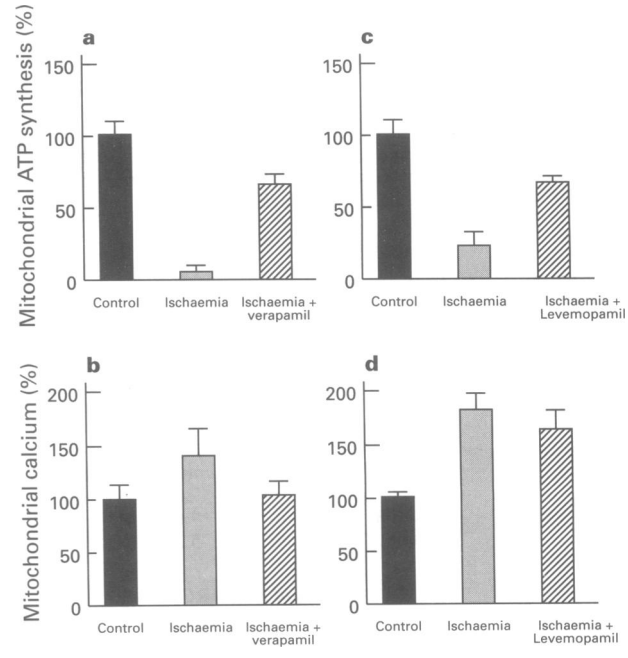

Figure 4 Comparative effects of verapamil (V) (a,b) and levemopamil (L) (c,d) on ATP synthesis and calcium concentration of renal cortical mitochondria at 6 h after reflow following 1 h of complete occlusion of the left renal artery. Solid columns represent values of right intact kidneys; values of left ischaemic kidneys from V- and L-treated rats are represented by stippled and hatched bars, respectively (means ± s.e.mean); data for V-treated rats are taken from Mohaupt & Kramer (1989/1990) and Kramer *et al.* (1983).

in left kidneys, more sodium was filtered by the intact right kidney. The increased amount of sodium delivered to the ascending limb of the loop of Henle probably resulted in a similar effect on the enzyme that we previously observed in acutely saline-loaded rats (Kramer & Gonick, 1974) and that others also found after uninephrectomy (Scherzer *et al.*, 1985).

Oxidative phosphorylation in cortical mitochondria as reflected by the ADP/O ratio and the rate of ATP synthesis were markedly impaired. This defect may be caused in part by the rise in intramitochondrial calcium concentration (Cheung *et al.*, 1986; Schrier *et al.*, 1987) which we observed in ischaemic kidneys. Thus, although mitochondria can buffer relatively large amounts of calcium without loss of metabolic activity (Bonventre, 1988), a rise in intracellular calcium may trigger other mechanisms of cell injury, e.g. activation of proteases and production of reactive oxygen species of which the hydroxyl radical ($OH^{\cdot}$) is known to be the most toxic (Baud & Ardaillou, 1986; Ratych & Bulkley, 1986; Canavese *et al.*, 1988).

Mitochondrial function was well preserved in L-treated rats. This may not be attributed solely to the drug's relative minor attenuation of the rise in intramitochondrial calcium content. Some of the effects of L could thus be due to properties other than that of calcium channel blockade.

In vitro, binding of L to calcium channels in membranes from rat cerebral cortex was found to be quantitatively similar to that of its parent compound verapamil but was without stereospecificity (Hofmann *et al.*, 1989). In rat aortic strips L revealed lesser calcium channel blocker activity than verapamil but its 5-HT-antagonist efficacy was an order of magnitude greater than that of verapamil (Hofmann *et al.*, 1989). This is supported by the finding that L revealed a stereoselective high affinity to the 5-HT_2-receptor in membranes from rat cerebral cortex, i.e. displacing [^{3}H]-ketanserin, a specific 5-HT_2-receptor blocker, with a K_i of 4.4 nmol l^{-1} as compared to 177 nmol l^{-1} for verapamil.

In vivo, L revealed a dose-related protection, i.e. prolonged survival time, from acute cerebral ischaemia of the rat in contrast to verapamil which had only a weak protective effect (Hofmann *et al.*, 1989; Defeudis, 1989). Such effects of L may apply to the kidney as well, as suggested by our results of the present and previous studies (Kramer *et al.*, 1983; Mohaupt & Kramer, 1989/1990). Most strikingly, we found that L, in contrast to verapamil, protected cell ATP synthesis apparently independently of its effects on mitochondrial calcium accumulation (Figure 4).

It is conceivable that in the present experimental setting unilateral occlusion of the renal artery resulted in aggregation of activated platelets within the renal vasculature which then released 5-HT. Therefore, tissue protection by L, as observed in the present study, may be related more to its action as a 5-HT_2-antagonist than as a calcium channel blocker. In support of this possibility the 5-HT_2-antagonist, ketanserin, was found in the normal rat to preserve RBF and GFR in ranges of perfusion pressure far below those of normal autoregulation of RBF (Lameire *et al.*, 1990). These authors also found a protection of renal function in experimental ischaemic ARF by ketanserin (Smollich *et al.*, 1990).

Alternatively, mediation of the protective effects of L through preventing enhanced synthesis of cyclo-oxygenase products (Blackshear *et al.*, 1986; Knauss & Abboud, 1986; Mené *et al.*, 1991) would be compatible with our previous observations that TXA_2 receptor blockade had a similar though quantitatively smaller protection in the ischaemic kidney after reflow (Kramer *et al.*, 1993).

Taken together, our present findings in this model of ischaemic ARF suggest the use of L as a tool to gain further insight into the role of 5-HT in the pathophysiological events of ischaemic ARF. Further detailed studies are also required to evaluate the potential role of compounds with 5-HT_2-receptor blocking properties in preventing or ameliorating circulatory ARF in experimental and clinical settings.

Some of these results were presented at the 12th International Congress of Nephrology 1993 in Jerusalem, Israel. Levemopamil was a gift from Knoll AG, Ludwigshafen, Germany. The authors thank Dr H. Schulebusch, Dept of Gynecology, for his help in measuring mitochondrial calcium and Professor Dr H. Lehmann, Knoll AG, for valuable support. The authors also acknowledge the excellent technical assistance of Mrs H. Stelkens.

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(Received October 19, 1994
Revised November 20, 1995
Accepted November 27, 1995)