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# Impairment of cellular redox status and membrane protein activities in kidneys from rats with ischemic acute renal failure

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**Abstract**

Cellular redox status and membrane protein activities were analyzed in kidneys from rats with ischemic acute renal failure (ARF). ARF was induced by clamping the left renal artery for 50 min. A parallel group of control animals was processed. In the ischemic group urea plasma levels were statistically increased as compared with the control group. Studies employing whole kidney homogenates revealed that ischemia produces an increment in lipid peroxidation levels and a reduction in glutathione concentration and in superoxide dismutase and glutathione peroxidase activities. Since lipid peroxidation may alter the function of membrane proteins we determined succinate cytochrome *c* reductase (SuccR), sodium-potassium ATPase (Na-K-ATPase), glucose-6-phosphatase (G-6-Pase) and alkaline phosphatase (ALP) activities in whole renal homogenates. Only G-6-Pase and ALP activities were modified by ischemia. Since ALP is a brush border membrane (BBM) enzyme and BBM is one of the main target structures in ARF, we assessed some parameters of BBM functionality. ALP, γ-glutamyl transferase (γ-GT) and 5′-nucleotidase (5′-NT) showed diminished activities in BBM from ischemic kidneys. Ischemia also modified the $V_{max}$ of paraaminohippuric acid (PAH) uptake without altering $K_m$. An increment of lipid peroxidation and membrane fluidity in BBM was observed after the treatment. Total membrane proteins and protein recoveries in BBM were similar in both experimental groups. Sialic acid and sulfhydryl levels were similar in BBM from ischemic kidney and control ones. In summary, ARF induced by renal artery clamping for 50 min takes place with a significant increase in urea plasma levels. A decrease in the antioxidant defense system is detected. This induces lipid peroxidation in whole renal tissue, which may justify the diminished activities of some membrane enzymes such as G-6-Pase and ALP. A specific analysis of BBM function reveals a significant increment of lipid peroxidation which may be the cause of an increased membrane fluidity. This latter parameter might be, at least in part, responsible for the damaged function of apical ALP, 5′-NT, γ-GT and PAH carrier. 

*Keywords:* Ischemia; Acute renal failure; Membrane enzyme; Antioxidant defense system; Paraaminohippuric acid uptake; Brush border membrane vesicle; Membrane fluidity; Lipid peroxidation

## 1. Introduction

Ischemic injury occurs when a reduction in blood flow decreases the delivery of oxygen and substrates to a level inadequate to maintain cellular energy status. Cellular depletion of ATP, the initial pathophysiologic event and hallmark of ischemic injury, leads to a series of morphologic, biochemical and physiologic derangements [1,2]

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Renal ischemia is a major cause of acute renal failure (ARF). Ischemic renal failure occurs following an episode of severe hemorrhagic shock, endotoxic sepsis, thermal burns, or transplantation surgery [3,4].

The ARF syndrome is characterized by the rapid (hours to weeks) decline of the glomerular filtration rate and the retention of nitrogenous waste products such as blood urea nitrogen (BUN) and creatinine. The measure of these products is likely to remain as the principal method of ARF diagnosis [5–7].

The proximal tubule is the primary site and the most susceptible to cellular damage induced by ischemia. The most sensitive cell to in vivo ischemic injury in the kidney has been shown to be in the S3 segment [6,8]. The most extensively studied ARF model is provided by total cessation of renal blood flow (typically by renal artery clamping). This model defines the multiple factors that may potentially contribute to the pathophysiology of ischemic renal injury, which makes it a very valuable tool. This work pursued the following goals:

1. to obtain an experimental model of an early stage of ischemic ARF which may be clinically detected by an increment of BUN. The left renal artery of the rat was clamped during different periods of time and BUN was measured simultaneously.
2. to further characterize the mechanisms responsible for ischemia induced injury of epithelial cells in this model. In order to achieve this, we analyzed the following items in renal cortical tissue:
   2.1. redox cellular status in whole homogenates: thiobarbituric acid reactive species (TBARS), glutathione concentrations (GSH); superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) activities.
   2.2. activity of some membrane enzymes in whole homogenates: succinate cytochrome *c* reductase (SuccR), sodium-potassium ATPase (Na-K-ATPase), glucose-6-phosphatase (G-6-Pase) and alkaline phosphatase (ALP).
   2.3. function of brush border membranes (one of the primary sites of ischemia induced cellular injury [6,8]) by assaying:
   - protein function: e.g. enzyme activities: alkaline phosphatase (ALP), γ-glutamyl transferase (γ-GT), 5′-nucleotidase (5′-NT) and transport of paraaminohippuric acid (PAH)
   - lipid function assayed as lipid peroxidation levels and membrane fluidity
   - in order to obtain preliminary evidence about the quality of proteins inserted in brush border membrane, sialic acid and sulfhydryl contents were assayed.

## 2. Materials and methods

Male Wistar rats (110–130 days old) weighing 300–350 g were used throughout the study. Animals were allowed free access to a standard laboratory chow and tap water, and housed in a constant temperature and humidity environment.

Animals were anesthetized with sodium thiopental (70 mg/kg b.w., i.p.). In order to select an adequate time to induce acute ischemia, a non-traumatic vascular clamp was placed around the left renal artery for 0, 10, 20 and 50 min. To avoid reflows, the clamp was left in place until the kidney was removed. After this procedure, a blood sample was obtained by cardiac puncture in order to determine plasma urea levels. Once 50 min was selected as the optimal time to induce acute renal failure, kidneys were excised and divided for: (a) histopathology studies, (b) assessment of GSH and TBARS levels and enzyme activities (SOD, GSH-Px, CAT, SuccR, Na-K-ATPase, G-6-Pase, ALP); (c) studies with brush border membrane vesicles (BBMV). BBMV were employed to determine enzyme activities (ALP, γ-GT, 5′-NT), PAH transport studies, TBARS levels, fluorescence polarization studies, sialic acid and sulfhydryl content. A parallel group of control rats was also processed.

### *2.1. Histopathological studies*

Histopathology of kidneys was performed after fixing in 4% neutral buffered formalin for 2 and 4 h and embedding in paraffin, then 4 μm thick sections were processed for routine staining with hematoxylin and eosin.

### 2.2. Assessment of GSH and TBARS levels and enzyme activities

In order to analyze GSH and TBARS levels and enzyme activities, kidney cortices were cut off ($n=4$ for the control group and $n=6$ for the ischemic group) and homogenized in 30 volumes (w/v) 250 mM sucrose, 10 mM HEPES-Tris (pH 7.40).

Determination of non-protein sulfhydryl (95% GSH) was performed as described by Ellman [9]. TBARS were quantitated according to the method of Ohkawa et al. [10]. Malondialdehyde (MDA), an end product of lipid peroxidation, reacts with thiobarbituric acid (TBA) to yield a colored substance. Measurement of MDA by TBA is one of the most widely used methods for assessing lipid peroxides.

SOD activity was assayed by its ability to inhibit superoxide radical dependent reactions [11]. The reduction rate of cytochrome *c* by superoxide radicals was monitored at 550 nm using the xanthine-xanthine oxidase system as the source of superoxide radical. One unit of SOD is defined as the amount of SOD in the homogenate required to inhibit 50% of the rate of cytochrome *c* reduction.

GSH-Px was assayed by continuous monitoring of GSSG formation. GSSG formed during the glutathione peroxidase reaction was instantly and continuously reduced by an excess of glutathione reductase activity providing for a constant level of GSH. The concomitant oxidation of NADPH was monitored photometrically [12]. The rate of change of absorbance during the conversion of NADPH to NADP was recorded at 340 nm for 5 min. GSH-Px activity was expressed as μmol of NADPH oxidized to NADP/min/mg protein.

CAT activity measurement was based on the titrimetric determination of $H_2O_2$ with $KMnO_4$ [13]. The activity is expressed as *k*/min/mg protein, where *k* is the first order rate constant.

SuccR was measured spectrophotometrically at 30°C by following the reduction of cytochrome *c* at 550 nm [14].

Na-K-ATPase activity was estimated as the difference between the amounts of inorganic phosphate released in the absence (total ATPase) and in the presence (Mg-ATPase) of ouabain [15].

G-6-Pase activity was measured as described by Sottocasa et al. [14].

ALP activity was assayed employing a Wiener commercial kit.

The release of inorganic phosphate was measured according to Widnell et al. [16]. Proteins were analyzed by the method of Smith et al. [17].

### 2.3. Studies with brush border membrane vesicles

#### 2.3.1. Preparation of brush border membrane vesicles from kidney cortex

BBMV were isolated from renal cortex by Mg/EGTA precipitation as described by Ohoka et al. [18]. The BBMV obtained were resuspended in 250 mM sucrose, HEPES-Tris 10 mM, pH 7.40. Aliquots of the membranes were frozen in liquid nitrogen and stored immediately at −70°C until use. Three preparations were made for the control group and three for the ischemic group. Each preparation represented the left renal cortical tissues from six animals.

#### 2.3.2. Enzyme assays

The purity of BBMV preparation was monitored by measuring the specific activity of ALP, SuccR, G-6-Pase, and Na-K-ATPase as described above. γ-GT activity in BBMV was determined kinetically at 405 nm employing γ-glutamyl-*p*-nitroanilide as substrate using a commercial kit (γ-GT Test, Wiener Lab, Argentina). 5′-NT activity in BBMV was also assayed employing a Wiener commercial kit.

#### 2.3.3. PAH transport studies

PAH uptake by BBMV was measured by the rapid filtration technique using a Millipore vacuum filtration system and nitrocellulose membranes with a pore size of 0.45 μm (HAWP 0.24, Millipore, Bedford, MA, USA) as reported by Ohoka et al. [18]. All filters were soaked and presaturated overnight in 1 mM unlabelled PAH in 100 mM NaCl, 1 mM HEPES-Tris, pH 7.40 to minimize non-specific binding of radioactivity. The degree of non-specific binding to the filter was about 0.02%. BBMV were rapidly thawed at 37°C and preincubated in 100 mM sucrose, 100 mM K-gluconate, 10 mM HEPES-Tris pH 7.40 at 37°C for 1 h. The reaction was started by adding 25 μl of vesicles (125–200 μg protein) to 300 μl of incubation medium at 37°C. The medium contained (mM) 100 sucrose, 100 K-gluconate, 10 HEPES-Tris, pH 7.40, 10 μCi $^3$H-PAH to which

unlabelled PAH was added to yield final concentrations. After a predetermined incubation time, the uptake was terminated by diluting the samples to 1.0 ml with ice-cold stop solution (150 mM KCl, 20 mM HEPES-Tris pH 7.40). The diluted samples were immediately filtered, then the filters were washed three times with 3 ml ice-cold stop solution. Radioactivity in the filters was measured in a liquid scintillation spectrometer counter. Uptake at time zero was assessed by simultaneously mixing 1 ml of cold stop solution with 25 μl of membranes and 300 μl of the isotope-containing buffer. The diluted sample was immediately filtered and washed three times with 3 ml of stop solution. The 'uptake' at time zero was subtracted from that obtained at the same PAH concentration after incubating at 37°C during a predetermined incubation time.

#### 2.3.4. Fluorescence polarization measurements in BBMV

The fluidity of brush border membranes was assessed by measuring the steady-state fluorescence anisotropy of 1,6-diphenyl-1,3,5-hexatriene (DPH) [19,20]. DPH was dissolved in tetrahydrofuran at a concentration of 1 mM and diluted 1000-fold in 10 mM HEPES-Tris pH 7.40. BBMV (270 μg protein) were incubated for 1 h at 25°C with DPH (final concentration 1 μM). The DPH was excited at 366 nm and emission was observed at 433 nm at 25°C. The fluorescence anisotropy was calculated using the equation $r=(I_{\|}-GI_{\perp}/I_{\|}+2GI_{\perp})$ where $I_{\|}$ and $I_{\perp}$ are the fluorescence intensities observed with the polarized light emitted parallel and perpendicular, respectively, to the excitation polarizer. $G$ is the correction factor for the optical system, given by the ratio of vertically to horizontally polarized emission components when the excitation light is polarized in the horizontal direction [21].

#### 2.3.5. Sialic acid content in BBMV

Sialic acid was quantitated employing the thiobarbituric assay [22]. The color of the chromophore obtained was intensified by the addition of dimethyl sulfoxide [23]. The exposed sialic acid were assayed before and after the treatment with Triton X-100.

#### 2.3.6. Measurement of sulfhydryl content in BBMV

The membranes (0.1 mg protein/ml) were incubated with 10 mM 5,5′-dithiobis (2-nitrobenzoate) (DTNB) in 50 mM Tris-HCl buffer (pH 8.0) for 10 min at 37°C in the presence of 3% SDS. The amount of DTNB-reactive SH groups was determined using the molar extinction coefficient of $1.36\times10^{4}$ $M^{-1}$ $cm^{-1}$ at 412 nm [9].

#### 2.3.7. Others determinations

TBARS levels in BBMV were assayed as described above. Proteins were also analyzed by the method of Smith et al. [17].

### 2.4. Materials

Chemicals were purchased from Sigma (St. Louis, MO, USA) and were analytical grade pure. $^{3}$H-PAH was obtained from DuPont-New England Nuclear.

### 2.5. Statistical analysis

The results are expressed as means ± S.E.M. The comparison between the control and ischemic groups was made using the unpaired $t$-test. $P<0.05$ was regarded as statistically significant. The results obtained with BBMV are expressed as means ± S.D. of at least three different experiments performed in at least three different vesicle preparations for each experimental group. Kinetic data were fitted by the Michaelis-Menten model with a non-linear, least square criterion using the Enzfitter program (Elsevier Biosoft).

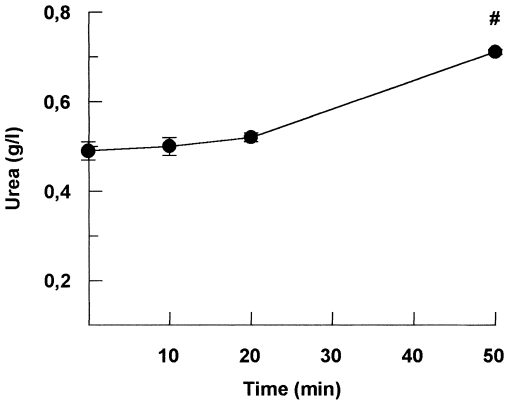


Fig. 1. Urea plasma levels 0, 10, 20 and 50 min post left renal artery clamping.

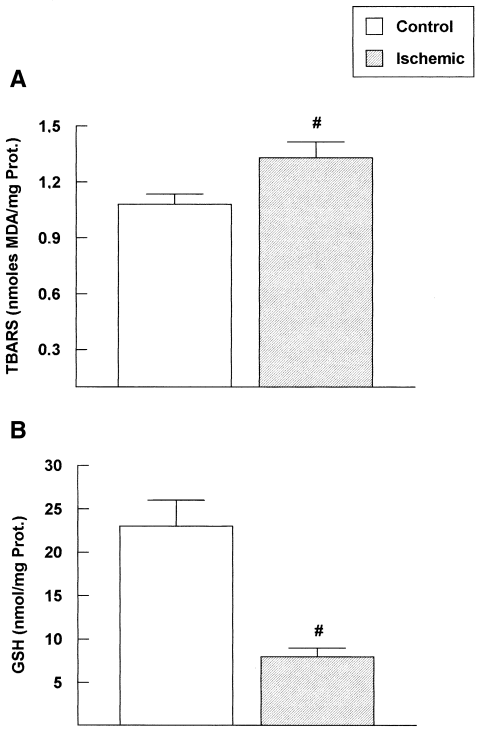


Fig. 2. Thiobarbituric acid reactive species (TBARS, A) and glutathione levels (GSH, B) in control ($n=4$) and ischemic ($n=6$) kidneys. Results are expressed as means ± S.E.M. $^{\#}P<0.05$ control vs. ischemic group.

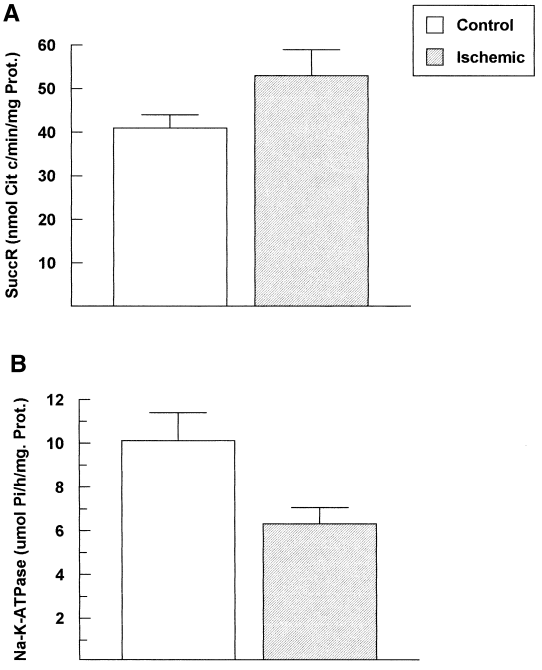


Fig. 3. (A) Succinate cytochrome *c* reductase (SuccR) activity in control ($n=4$) and ischemic ($n=6$) kidneys. (B) Sodium-potassium-ATPase (Na-K-ATPase) activity in control ($n=3$) and ischemic ($n=3$) kidneys. Results are expressed as means ± S.E.M.

## 3. Results

Fig. 1 shows urea plasma levels after different periods of left renal artery clamping. Only after 50 min of ischemia was a statistically increment in this parameter observed.

Histopathological studies revealed a corticomedullary congestion and some subcapsular cytoplasmic degeneration of proximal convoluted tubules in ischemic kidneys.

Fig. 2A shows that ischemic kidneys presented higher levels of TBARS than control kidneys. Fig. 2B shows a significant decrease of GSH levels after the treatment.

The activities of antioxidant enzymes SOD, GSH-Px and CAT are summarized in Table 1. The ischemic situation caused a significant diminution in renal SOD and GSH-Px activities. CAT activity was stable in the face of the ischemic injury.

After the treatment no modifications were observed in SuccR and Na-K-ATPase (Fig. 3). ALP and G-6-Pase activities in whole homogenates from ischemic kidneys were statistically different from those observed in the control group (Fig. 4).

Table 1
Superoxide dismutase, glutathione peroxidase and catalase activities in control ($n=4$) and ischemic ($n=6$) kidneys

| | Superoxide dismutase (U SOD/mg protein) | Glutathione peroxidase (μmol/min/mg protein) | Catalase (*k*/min/mg protein) |
|---|---|---|---|
| Control kidneys | 148 ± 13 | 2.14 ± 0.13 | 3.48 ± 0.69 |
| Ischemic kidneys | 108 ± 4* | 1.08 ± 0.04* | 3.85 ± 0.15 |

Results are expressed as means ± S.E.M.
*$P<0.05$ control vs. ischemic group.

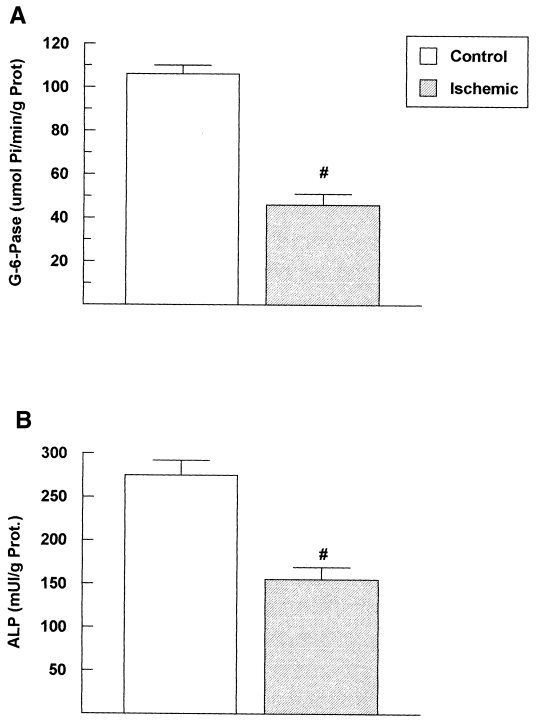


Fig. 4. Glucose-6-phosphatase (G-6-Pase, A) and alkaline phosphatase (ALP, B) activities in control ($n=4$) and ischemic ($n=6$) kidneys. Results are expressed as means ± S.E.M. $^{\#}P<0.05$ control vs. ischemic group.

Table 2 summarizes the specific activities and enrichment of marker enzymes, in both the homogenates and final brush border membrane isolated from control and ischemic kidneys. The specific activity of ALP in the brush border membrane was 15-fold higher than that in the homogenate in both experimental groups, indicating a similar purity of the membranes. However, the specific activity of ALP was reduced in both the homogenate and brush border membrane from ischemic kidneys. Contamination of brush border membranes by intracellular organelles was not modified, but specific activities of G-6-Pase (microsomal marker) was reduced in homogenates from ischemic kidneys. Neither specific activity nor SuccR (mitochondrial marker) contamination was altered by ischemia. No significant difference between the control and ischemic kidneys was observed with regard to the specific activities of Na-K-ATPase in the homogenates and final brush border membrane. This agrees with the results of Maeda et al. [24] and Kim et al. [25], but not with the data reported by Molitoris et al. [26]. No differences were observed in total protein levels and protein recovery between groups. This fact indicates no modification in protein quantity after the treatment.

To estimate the relative proportion of right side out vesicles the exposed sialic acid was assessed before and after treatment with Triton X-100. The orientation of BBMV was 80 ± 1.3 and 78 ± 6.6% right side out in the control and ischemic groups respectively.

ALP, γ-GT and 5′-NT, marker enzymes of apical membranes, were sensitive to blood flow interruption (Table 3).

Ischemia decreased the initial PAH uptake compared to control (38 ± 5 vs. 77 ± 9 nmol PAH/mg

Table 2
Specific activities and enrichment of marker enzymes in rat renal cortex homogenates and brush border membrane vesicles isolated from control (C) and ischemic (Is) kidneys

| | Homogenates | Vesicles | Enrichment |
|---|---|---|---|
| Succinate cytochrome *c* reductase (nmol Cit/min/mg protein) | C: 43 ± 2 | C: 2.75 ± 0.68 | C: 0.063 ± 0.012 |
| | Is: 49 ± 4 | Is: 1.27 ± 0.09 | Is: 0.026 ± 0.009 |
| glucose-6-phosphatase (μmol Pi/min/mg protein) | C: 102 ± 2 | C: 96 ± 7 | C: 0.94 ± 0.08 |
| | Is: 56 ± 6* | Is: 80 ± 6 | Is: 1.43 ± 0.06 |
| Na-K-ATPase (μmol Pi/mg protein/h) | C: 10.12 ± 1.28 | C: 4.77 ± 1.66 | C: 0.44 ± 0.10 |
| | Is: 6.31 ± 0.74 | Is: 4.49 ± 3.52 | Is: 0.62 ± 0.49 |
| Alkaline phosphatase (mIU/mg protein) | C: 265 ± 19 | C: 3979 ± 232 | C: 15.09 ± 0.78 |
| | Is: 173 ± 15* | Is: 2590 ± 148* | Is: 15.10 ± 0.80 |
| Proteins (mg) | C: 941 ± 111 | C: 21.93 ± 1.89 | C: 0.024 ± 0.002 |
| | Is: 856 ± 139 | Is: 23.51 ± 4.00 | Is: 0.027 ± 0.003 |

Results are expressed as means ± S.D. from experiments carried out in triplicate in at least three different vesicle preparations for each experimental group.
*$P<0.05$ control vs. ischemic group.

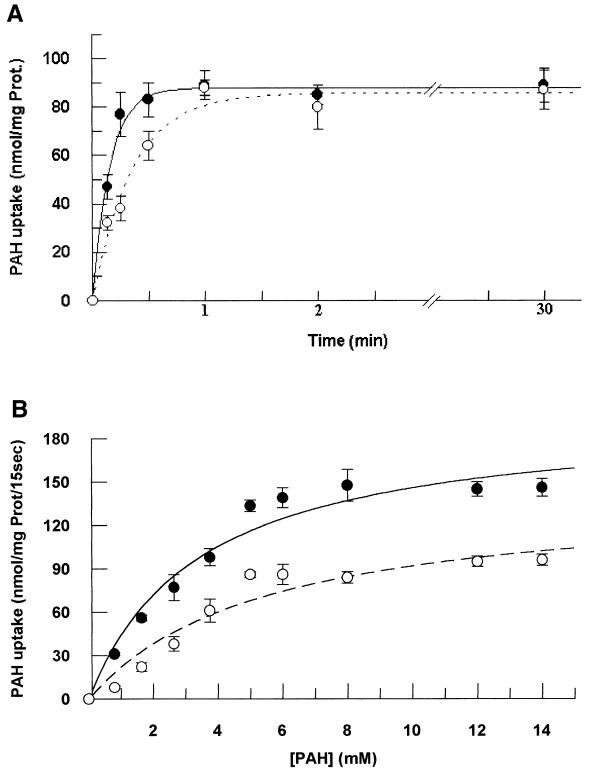


Fig. 5. PAH uptake in brush border membrane vesicles from control (●) and ischemic (○) kidneys. (A) Time course of PAH uptake. Membrane vesicles (25 μl, 125–200 μg of protein) were incubated for different times (0.8 s–30 min) at 37°C with 300 μl of incubation medium containing 2.63 mM $^{3}$H-PAH. (B) Kinetics of PAH uptake. Membrane vesicles (25 μl, 125–200 μg of protein) were incubated for 15 s at 37°C with 300 μl of incubation medium containing different concentrations of $^{3}$H-PAH (0.8–14 mM). Results are means ± S.D. from experiments carried out in triplicate in at least three different vesicles preparations for each experimental groups.

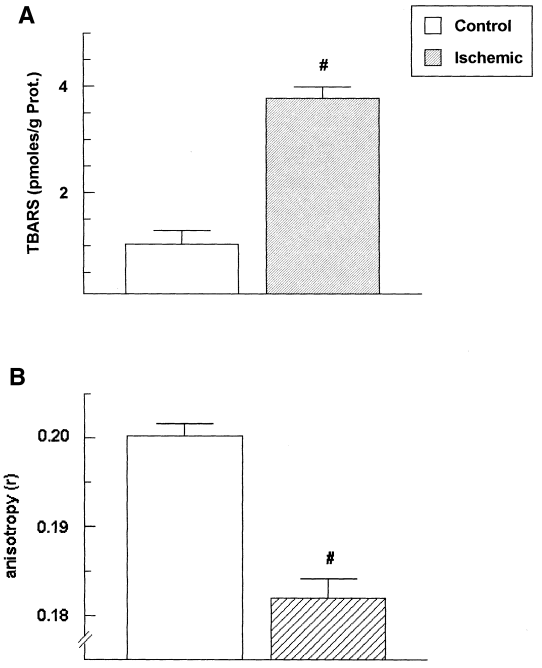


Fig. 6. Thiobarbituric acid reactive species (TBARS, A) and anisotropy values ($r$, B) in brush border membrane vesicles from control and ischemic kidneys. Results are means ± S.D. from experiments carried out in triplicate on at least three different vesicles preparations for each experimental group. $^{\#}P < 0.05$ control vs. ischemic group.

protein per 15 s, respectively, $P < 0.05$). The time course of $^{3}$H-PAH uptake by BBMV was determined over a period of 30 min. The uptake rose to an equilibrium plateau at 60 s and was constant thereafter in both groups. Uptake velocity was linear up to 15 s in the control group and up to 30 s in the ischemic group. Accordingly, unless otherwise indicated, the 15 s time was chosen to measure PAH uptake to fulfill the requirements for measuring linear transport. A statistical diminution in the initial uptake velocity up to 30 s was observed in ischemic BBMV (Fig. 5A). To determine whether this effect of ischemia was due to an alteration in the substrate

Table 3
Alkaline phosphatase, γ-glutamyltransferase and 5′-nucleotidase activities in brush border membranes from control and ischemic kidneys

| | Alkaline phosphatase (mIU/mg protein) | γ-Glutamyltransferase (IU/mg protein) | 5′-Nucleotidase (mIU/mg protein) |
|---|---|---|---|
| Control kidneys | 3979 ± 232 | 48 066 ± 2230 | 538 ± 36 |
| Ischemic kidneys | 2590 ± 148* | 35 043 ± 677* | 304 ± 14* |

Results are expressed as means ± S.D. from experiments carried out in triplicate in at least three different vesicle preparations for each experimental group.
*$P < 0.05$ control vs. ischemic group.

Table 4
Sialic acid and sulfhydryl residue content in brush border membrane vesicles isolated from control and ischemic kidneys

| | Sialic acid residues (nmol/mg protein) | Sulfhydryl residues (μmol/mg protein) |
|---|---|---|
| Control kidneys | 80 ± 3.69 | 3.29 ± 0.16 |
| Ischemic kidneys | 81 ± 4.36 | 3.64 ± 0.06 |

Results are expressed as means ± S.D. from experiments carried out in triplicate in at least three different vesicle preparations for each experimental group.

affinity ($K_m$) and/or the maximum velocity ($V_{max}$) of PAH transport, the initial rate (15 s) of PAH uptake was measured as a function of the external PAH concentrations (Fig. 5B). Ischemia decreased $V_{max}$ as compared with control (160 ± 2 vs. 214 ± 16 nmol PAH/mg protein per 15 s, respectively, $P < 0.05$). No variations were observed for $K_m$ (mM) (4.93 ± 0.19 for the control group vs. 5.49 ± 0.58 for the ischemic group).

Fig. 6 summarizes the effects of ischemia on TBARS and membrane fluidity in BBMV. Ischemia increased both TBARS levels and membrane fluidity.

In order to have a preliminary assessment of protein quality, we measured sialic acid content (since the negatively charged terminal sialic acid is an important residue of microvillus glycoproteins) and sulfhydryl content in BBMV. Neither sialic acid nor sulfhydryl residues changed with the treatment (Table 4).

## 4. Discussion

Ischemia involves cessation of circulation to a tissue so that metabolic substrates are not delivered and metabolic products are not removed. Renal ischemia is a major cause of ARF. ARF by definition means acute impairment of renal excretory function and is characterized by progressive azotemia [3–6]. In this work we obtained an experimental model of an early stage of ARF.

A number of processes have been implicated in the pathogenesis of oxygen deprivation-induced cell injury. These include disturbances of cell calcium metabolism, disruption of the cytoskeleton, depletion of adenine nucleotides, activation of phospholipases with production of toxic lipid metabolites, loss of cell volume and monovalent cation homeostasis and generation of free radicals [5–8].

Tissue hypoxia favors the univalent reduction of oxygen molecules that produces superoxide anions, hydrogen peroxide and hydroxyl radicals [1,2]. The main source of reactive oxygen species under hypoxic conditions is the xanthine oxidase reaction [27–30]. To protect the cell against toxic oxygen metabolites, there are several physiological defense mechanisms, such as SOD, GSH-Px, CAT and the tripeptide GSH [31,32]. The increment of TBARS in our experimental model, indicating the presence of lipid peroxidation in the tissue, may be the consequence of an increment in the formation of oxygen free radicals (generated by the xanthine-xanthine oxidase system) and/or an impairment of its metabolization since antioxidant defense systems are compromised. In this sense, it has been described that GSH-Px and SOD can be inactivated by free radicals [33]. Moreover, GSH levels in our experimental model were reduced as also described by others [34].

Lipid peroxidation results in the alteration of membrane properties, such as enzyme activities, solute transport and fluidity [31,32]. We observed a remarkable diminution of membrane enzymes activities, such as G-6-Pase and ALP, after ischemia. With respect to this point, it has been reported that altered membrane lipids from peroxidized microsomes are capable of inhibiting glucose 6-phosphatase [35].

Since ALP is a marker enzyme of the apical membrane (one of the target structures for renal ischemic injury [6,8,36]), we assessed the function of the brush border membrane. The activity of other apical membrane enzymes such as γ-GT and 5′-NT was also decreased, in addition to ALP, in BBMV from ischemic kidneys. In this connection, other authors have also reported a diminished activity of γ-GT and ALP after ischemia [24–26]. The organic anion carrier that has been described in brush border membrane transports many organic and inorganic anions such as urate, lactate, PAH, $OH^-$ and $Cl^-$ [37]. We measured PAH uptake. The reduction in $V_{max}$ indicates a diminution in the number of functional carrier units. In this regard, it has been reported that ischemia alters the transport properties of tetraethylammonium and D-glucose in rat renal brush border membranes [24,38]. Kim et al. [39] have reported that

tetraethylammonium but not PAH uptake in rabbit brush border membranes was reduced following 60 min of ischemia.

The effect of ischemia on the behavior of ALP, 5′-NT, γ-GT and the organic anion carrier might be explained by two possible mechanisms: alteration in the transmembrane mobility of protein units due to changes in the membrane lipid (composition and contents) and/or reduction of the number of protein units. We found a significant increment of TBARS specifically in BBMV from ischemic kidneys, indicating an increase of lipid peroxidation in these membranes. In this regard, Fujimoto and Fujita [40] have reported that lipid peroxidation alters PAH transport in rat kidney. It has been described that lipid peroxidation damages membrane fluidity [31,32,41]. In this sense, we found a significant increase of brush border membrane fluidity after ischemia. Membrane fluidity has been shown to affect numerous membrane functions including passive permeability, enzymatic activities and transport systems [42]. This effect can result from a direct action on the conformational changes required for the function of a given protein or may involve the ease with which proteins associate or dissociate [42,43]. On the other hand, Molitoris et al. [26] postulated a selective loss of brush border membrane proteins after ischemia. This fact would cause a reduction in the number of protein units. In contrast, we obtained evidence for a conservation in quality and quantity of protein content in renal apical membranes after 50 min of ischemia.

In summary, ARF induced by renal artery clamping during 50 min occurs with a significant increase in urea plasma levels. A decrease in the antioxidant defense system is found, which induces lipid peroxidation in whole renal tissue. This may explain the diminished activities of membrane enzymes such as G-6-Pase and ALP. A specific analysis of brush border membrane function reveals a significant increment of lipid peroxidation which might be the cause of an increased membrane fluidity. This latter parameter might be, at least in part, responsible for the damaged function of apical ALP, 5′-NT, γ-GT and PAH carrier.

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