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ULTRASTRUCTURAL MARKERS OF TUBULAR TRANSPORT IN ACUTE EXPERIMENTAL RENAL ISCHEMIA
EVALUATION OF THE PROXIMAL AND DISTAL TUBULES

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ABSTRACT

The study was carried out on rats. Electron micrographs of the kidney obtained from the proximal and distal tubules in acute experimental renal ischemia were analysed. Using stereological methods to estimate of ultrastructural markers of active transport (mitochondrial energy states), and passive transport (intercellular spaces and basal infolded channels) were evaluated. The results obtained indicated that in the proximal and distal tubules following acute renal ischemia, active transport was impaired as reflected by a lowering of mitochondrial energy states (similar to condensed). Widening of intercellular spaces and basal infolded channels was also observed, which suggests fluid stasis and, thus, impairment of passive transport.

KEY WORDS: ultrastructural markers, proximal and distal tubular transport, acute experimental renal ischemia

INTRODUCTION

Excretory processes are one of the main functions of the kidney. Due to partial filtration of the blood flowing through the kidneys, glomerular filtrate is formed, contents of which resemble that of blood plasma. Final urine significantly differs from the primary one. It is a consequence of reabsorption and active transport, particularly in the proximal tubule, where approximately 80% of glomerular filtrate is reabsorbed (Baldamus et al., 1972; Jacobson, 1982; Schafer and Burfuss, 1982 1982; Ottosen, 1984).

Active transport is realized with Na^+, K^+ - ATPase - driven pump (Györy and Kinne, 1971; Jergensen, 1980; Maunsbach et al., 1986; Seguro et al., 1989). Energy for ion pump functioning is supplied by mitochondria, which are numerous in the basal part of the proximal and distal tubular epithelial cells (Ullrich et al., 1974a; 1974b; Jergensen, 1980). Previous studies revealed that mitochondrial energy states can be recognized as ultrastructural markers of active transport as reflected by configurational states of these organelles (Baldamus et al., 1972; Welling et al., 1978). On the other hand, water and chloride reabsorption is a passive transport. Water shifts towards the space with higher ion concentration. Intercellular spaces and basal infolded channels of the proximal and distal tubular epithelial cells are accepted as ultrastructural markers of passive water transport (Baldamus et al., 1972; Welling et al., 1978).

In the presented studies we have decided to investigate ultrastructural markers

with stereological methods of the proximal and distal tubular transport in the kidney subjected to complete ischemia.

MATERIAL AND METHODS

The studies were performed on 15 male Wistar rats, weighing 200-250 g, fed with standard chow and water ad libitum. Considering circadian variations of renal function, material for investigations was always taken at 10°°a.m. The animals were divided into three groups, five rats each:

- group I - control animals,
- group II - rats with five-minute clamping of the renal artery followed by the release of the clamp,
- group III - rats with 45-minute clamping of the renal artery followed by the release of the clamp.

The animals were anasthetized with Brietal 100 mg/kg given intraperitoneally. Then, laparotomy was performed and the right renal artery was clamped for 5 minutes (group II) or 45 minutes (group III). After that time the right kidney was extirpated as soon as possible. In the control group (group I) the kidney was removed just after administration of anaesthesia.

In all groups specimens of the cortical and medullary part of kidney were take for electron microscopic examination as soon as possible. The sample was isotropic random sample i.e. all section orientations in space were equally likely, and it was a uniform random sample. The proximal and distal tubule epithelial cells were horizontal and vertical random sectioned in controls and the experimental samples. The systematic sampling with a random start was applied (Weibel et al., 1966). The specimens were cut into slices 1 mm thick and fixed with 2.5% glutaraldehyde in 2.0% paraformaldehyde in 0.1M cacodylate buffer, pH 7.4 supplemented with 0.2ml of 1M $MgCl_2$ per 100ml of the fixative, at 0-4° for 3 hours. The specimens were washed in 0.1M cacodylate buffer three times for 1 hour, and then postfixed in 2% osmium tetroxide for 1 hour. Next, they were washed in 0.1M cacodylate buffer again, then dehydrated in increasing concentrations of ethanol and propylene oxide. All specimens were embedded in Araldite and cut using LKB III Ultramicrotome. Following evaluation of semi-thin (1 µm) specimens one searched regions containing the proximal (S_1, S_2, S_3 segments) and distal tubules, which were then submitted to electron microscopic examinations. Ultrathin sections were stained with uranyl acetate and lead citrate for examinations in Philips EM 300 electron microscope.

Stereological analysis was performed using the techniques of Weibel et al., (1966). The embedded specimens were cut in ultrathin sections, and then micrographs were taken at a primary magnification of 27 000 x, one area from each cell. Twenty electron micrographs were taken from the proximal and twenty from distal tubular epithelial cells in each case. Thus, 100 electron micrographs were obtained for stereological analysis of mitochondria from the proximal and 100 from distal tubular epithelial cells. In total more than 1000 mitochondrial profiles were analyzed (from the proximal and distal tubules). Data for calculating the surface areas of mitochondrial membranes were obtained by superimposing a Weibel's lattice with 28 test lines (z = 0.370 µ) on the electron micrographs (18 x 24 cm) at a final magnification of 81 000 x. The following parameters were calculated:

- outer membrane surface area (S_{om}),
- inner membrane surface area (S_{im}),
- relative volume of the outer membrane (V_{om}),
- relative volume of the inner membrane (V_{im}),
- volume of the inner compartment (matrix) (V_{mat}),
- volume of the outer compartment (V_{oc}).

Stereological analysis of intercellular spaces and basal infolded channels was carried out using the Weibel's technique (Weibel et al., 1966). Micrographs of ultrathin sections were taken at a primary magnification of 10 400 x . In each case 10 electron micrographs from different specimens were taken from the proximal and distal tubules. Stereological analysis was performed using Weibel's lattice with 45 lines and 90 test points. The lattice was superimposed in electron

micrographs (18 x 24 cm) at a final magnification of 31 200 x. The relative volume of the space (V_v) and relative surface area of the space (S_v) were calculated. Morphometric examinations of mitochondria, intercellular spaces and basal infolded channels were carried out according to the methods described in previous reports (Kidawa et al., 1988a; 1988b). Statistical analysis included calculations of arithmetic means, standard deviations, standard errors of the means and variation coefficients. Moreover, distributions of individual samples were compared. Depending on whether the distributions were normal and variances were equal or not, one of the following tests was used: unpaired Student's test (SN), Satterwhite's test (SW), Wilcoxon's test (WI). All calculations were performed by means of SM4A computer using CMS conversational mode in Fortran. Computer automatically chose optimal statistical test during comparisons of distributions. Critical value of the significance level was determined as a number within the interval (0.01; 0.1). The difference was recognized as statistically significant, when < 0.05.

RESULTS

Visual analysis of electron micrographs of the control group disclosed that mitochondria were in transitional steady close to orthodox configuration (Fig. 1).

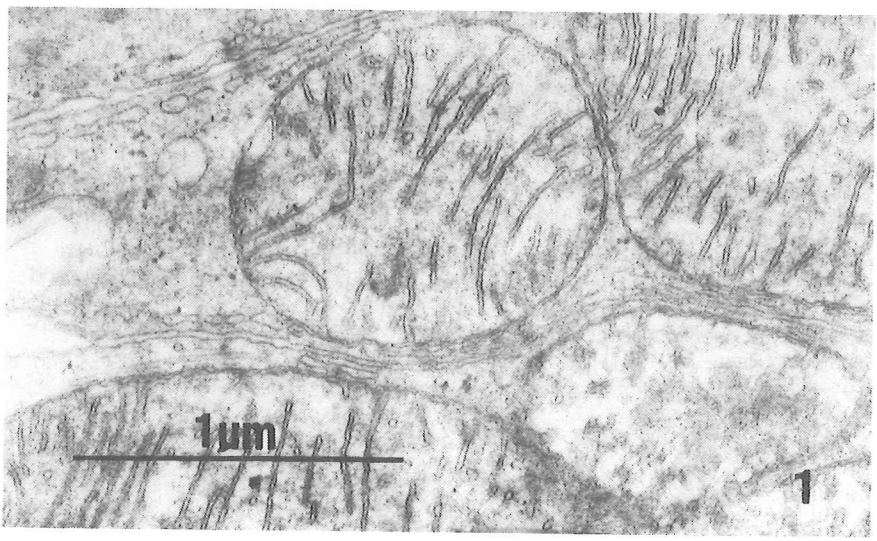

Figure 1. Mitochondria in the control group of the proximal tubules. The energy state - related configuration is close to orthodox.

In groups II and III epithelial cell mitochondria in the proximal tubules were significantly different from those found in the control animals and they were in a metabolic state - related configuration which was close to condensed configuration (Fig. 2).

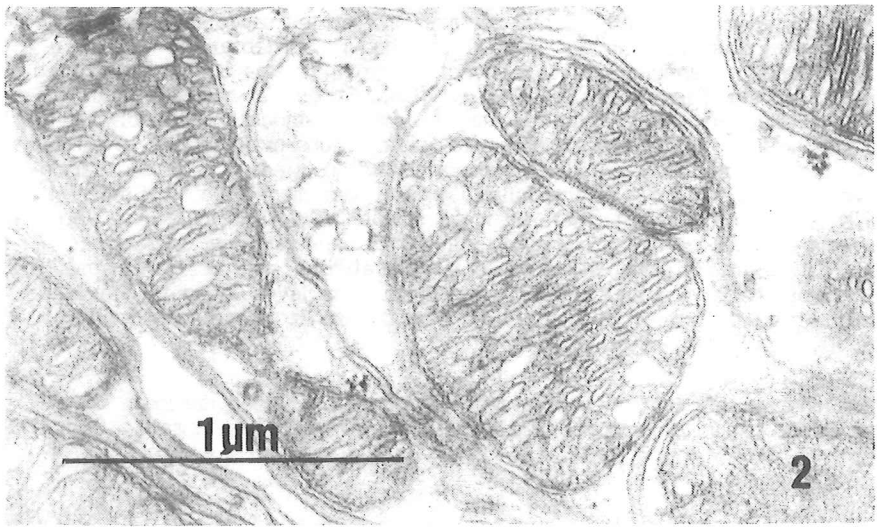

Figure 2. Mitochondria of the proximal tubules following 45-minute ischemia (group III). The energy state - related configuration is close to condensed.

During visual analysis of electron micrographs in the distal tubules presenting mitochondria of the control group it was found that mitochondria had transitional configurations close to orthodox.

In groups II and III mitochondria of the distal tubular epithelial cells were significantly different from those in the control group during visual analysis and they were in condensed configuration.

Directions of changes observed in visual analysis were confirmed by stereological analysis (Figs. 3a, 3b, and 4).

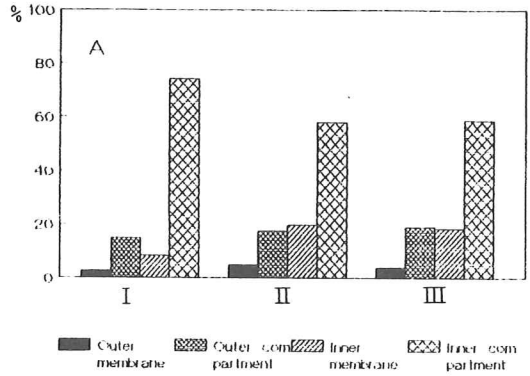

Figure 3a. Stereological analysis of the proximal tubular epithelial cell mitochondria in the control group (I) and following ischemia (group II and III).

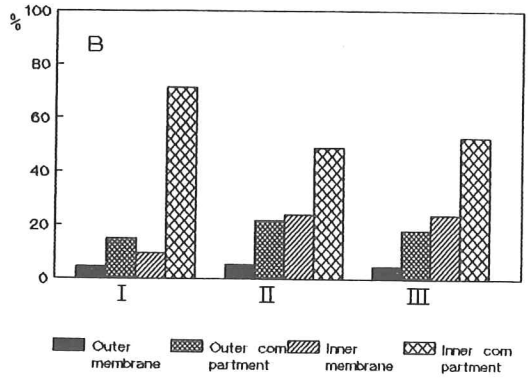

Figure 3b. Stereological analysis of the distal tubular epithelial cell mitochondria in the control group (I) and following ischemia (group II and III).

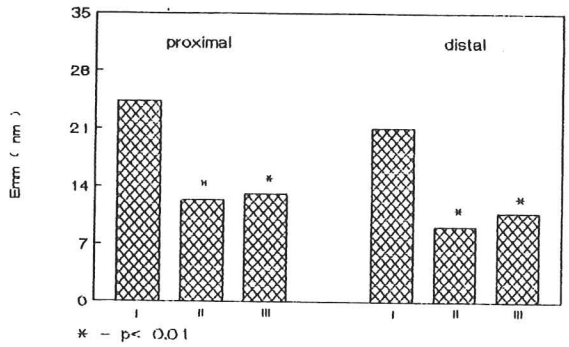

Figure 4. Statistical intergroup comparisons of the results of stereological analysis of the proximal and distal tubular epithelial cell mitochondria. Partition coefficient (E_{mm}) = inner compartment / inner membrane surface area.

In the proximal tubules of groups II and III outer compartment colume (V_{oc}) was statistically significantly increased in relation to that found in the control group. However, inner compartment volume (V_{ic}) significantly decreased from 0.74% ± 0.06% in group I to 0.58% ± 0.07% in group II and to 0.58% ± 0.07% in group III. The differences were statistically significant (Fig. 3a). Partition

coefficient of inner compartment per inner membrane surface area (E_{mm}) significantly decreased (Fig. 4).

In the distal tubules outer compartment volume (V_{oc}) significantly increased from 14.% ± 0.05% in group I (control) to 0.21% ± 0.04% in group II and 0.18% ± 0.04% in group III (Fig. 3b). Inner compartment volume (V_{mat}) significantly decreased from 0.71% ± 0.07% in group I to 0.49% ± 0.06% in group II and 0.52% ± 0.07% in group III (Figs. 3b and 4). Partition coefficient of inner compartment per inner membrane surface area (E_{mm}) was 21 ± 8 nm in group I and it significantly decreased to 9 ± 3 nm in group II and 10 ± 4 nm in group III (Figs. 3b and 4).

Stereological analysis of intercellular spaces and basal infolded channels revealed significant differences between the control group (I) and experimental groups (II and III). Significant differences was found both in the proximal (Figs. 5, 6) and distal tubular epithelial cells.

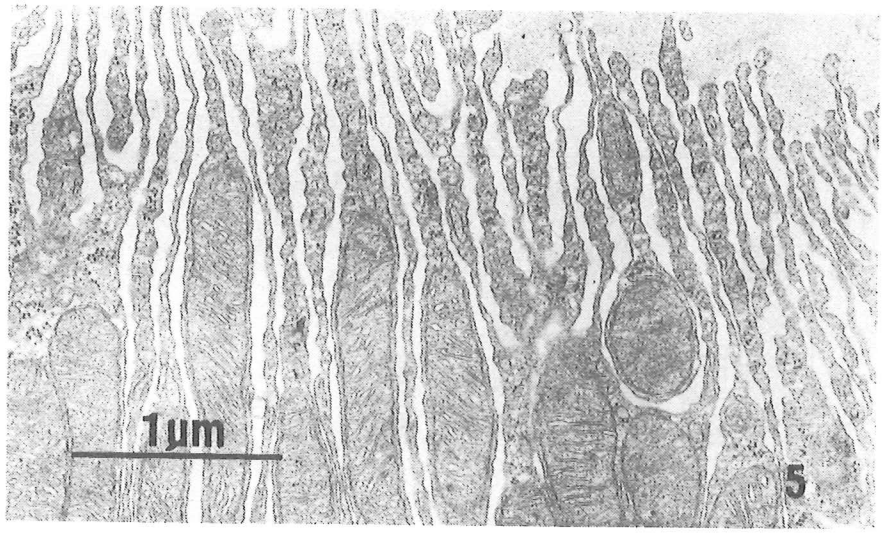

Figure 5. Intercellular spaces of the proximal tubular epithelial cells in the control group (I).

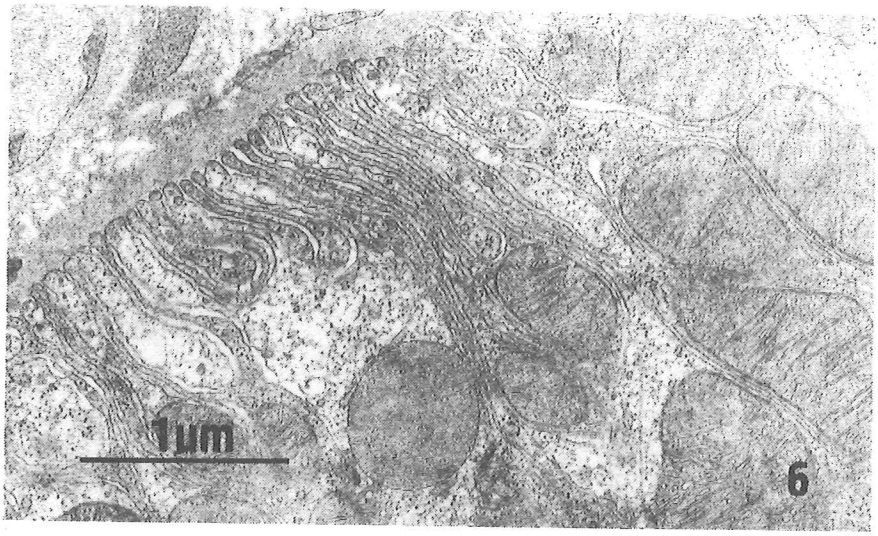

Figure 6. Intercellular spaces of the proximal tubular epithelial cells following 5-minute renal ischemia (group II). A significant narrowing of the intercellular spaces is seen in comparison with group I.

Stereological investigations of the intercellular spaces and basal infolded channels disclosed significant differences between the control group and experimental groups.

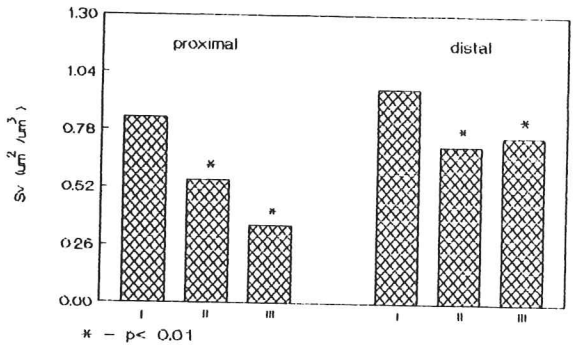

Figure 7. Relative surface areas of the intercellular spaces and basal infolded channels of the proximal and distal tubules.

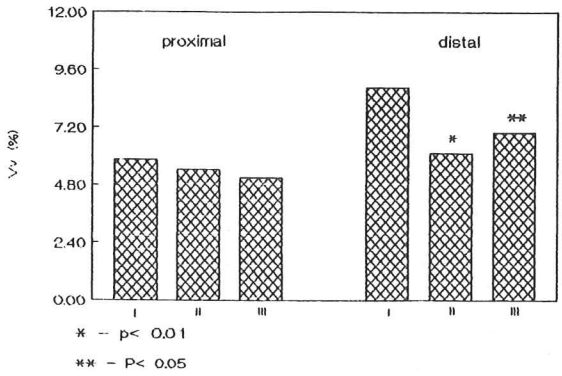

Figure 8. Relative volume of the intercellular spaces and basal infolded channels of the proximal and distal tubules.

In relation to the control group relative surface areas of intercellular spaces and basal infolded channels of the proximal tubules decreased by 34% following five-minute renal ischemia and by 58% following 45-minute ischemia (Fig.7). A similar direction of changes was observed with regard to relative volumes of these structure (Fig. 8).

In the distal tubules relative surface areas of the intercellular spaces and basal infolded channels also significantly (p 0.01) decreased in the experimental groups in comparison with the control group (Fig. 7). Relative volumes of these spaces significantly decreased from 8.84% ± 5.45% in group I to 6.11% ± 4.12% in group II and 6.99% ± 4.29% in group III (Fig. 8).

DISCUSSION

Induction of renal ischemia for 15-90 minutes is one of the methods for development of acute experimental renal failure (Tanner et al., 1973; Frega et al., 1976; Donohoe et al., 1978; Brezis et al., 1984). Transient renal ischemia is one of the principle causes of acute renal failure. Due to transient complete ischemia glomerular and tubular reabsorption become significantly impaired. These disturbances have a well-defined mitochondrial equivalent in the renal structure. In our studies we have evaluated ultrastructural alterations in the proximal and distal tubular epithelial cells in the very early phase of ischemia.

Previous observations disclosed that disturbances in the tubular reabsorption may be evaluated by means of ultrastructural markers of active and passive transports (Cieciura et al., 1984; Kidawa et al., 1988a; 1988b). Investigations of other authors proved significant deteriorations of renal tubular functions following acute experimental ischemia (Frega et al., 1976; Myers et al., 1984; Parekh et al., 1984; Kotowski et al., 1990; Okada and Morikawa 1990) and acute toxicity (Pfaller, 1982; Trump et al., 1989). Results of our investigations revealed also significant disturbances in ultrastructural markers of active and passive transport in the proximal and distal tubular epithelial cells following

acute ischemia. They make an attempt to evaluate energy system in ion pumps by means of the determination of mitochondrial energy states. It has been proven by many authors that changes in the mitochondrial configuration reflect their energy states (Chance and Williams, 1955; Hackenbrock, 1966; 1968; Cieciura et al., 1979; Pfaller, 1982). Accuracy of such an evaluation can be increased by the use of stereological methods (Pfaller, 1982). Data obtained from the stereological analysis of the proximal and distal tubular mitochondria indicate significant differences between experimental and the control groups with regard to surface areas and volumes of outer and inner membranes as well as partition coefficients for inner and outer compartments. The results suggest that following complete renal ischemia mitochondrial configuration change towards condensed or low-energy state. Due to renal ischemia contribution of mitochondria to active transport is markedly lower, which is suggested by changes in the partition coefficient of inner compartment. This coefficient enables to demonstrate differences in mitochondrial components such as membranes and compartments (Cieciura et al., 1979). In comparison with the control group its value was statistically significantly lower following renal ischemia lasting 5 and 45 minutes. Our results are consistent with observations of other authors (Bane et al., 1974; Frega, 1979; Harvig et al., 1980; Matthys et al., 1983; Brezis et al., 1984; Mason et al., 1984; Wilson et al., 1984; Seguro et al., 1989; Kotowski et al., 1990; Okada and Morikawa, 1990). For the evaluation of passive water transport, volumes and surface areas of the intercellular spaces and basal infolded channels of tubular epithelial cells were determined. Detailed morphometric investigations revealed that following transient renal ischemia intercellular spaces and basal infolded channels became narrowed, which may testify to the inhibition of passive water transport. In the proximal tubules relative surface areas of the intercellular spaces and basal infolded channels were found to decrease by 34% and 58% following 5-minute and 45-minute renal ischemia, respectively. In the distal tubules similar results were obtained. These results are comparable to those obtained by Kidawa et al., (1988a; 1988b) with ultrastructural markers of tubular transport in experimental diabetes insipidus and ethylene glycol poisoning.

The results of our morphometric investigations seem to suggest that transient, even short-term, complete renal ischemia induces the inhibition of active and passive tubular transport.

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