ULTRASTRUCTURAL MARKERS OF TUBULAR TRANSPORT IN EXPERIMENTAL DIABETES INSIPIDUS

L Cieciura, Z Kidawa, B Jaszczuk-Jarosz, K Trznadel, J Konopacki
2nd Clinic of Internal Diseases, Military Medical Academy, Łódź, Poland

Summary

Stereological analysis of changes in tubular transport markers in diabetes insipidus has been undertaken. Intracellular spaces and basal infolded channels were considered as the markers of water transport, while mitochondrial metabolic steady states were considered as the active transport markers. Transmission electron microscopic observations revealed morphometric differences in the surface area and volume of intercellular space and basal infolded channels in the distal tubules. Stereological markers of mitochondrial metabolic states demonstrated significant differences in the distal tubules between diabetes insipidus and control groups. In diabetes insipidus the volume and surface area of intercellular spaces and basal infolded channels in the distal tubules were decreased and the mitochondrial energy state was lowered.

Introduction

Recent reports [1] indicate that the epithelial cells of renal proximal and distal tubules contain considerable amounts of mitochondria. Comparison of changes in their configuration during normal and disturbed reabsorption may be a basis for elaboration of a method which would be helpful in the evaluation of disturbances in the ultrastructural markers of active tubular transport. Stereological methods enables the determination of the energy state of mitochondria and, thus, ion-pump function.

Water shifts are mainly associated with sodium transport and passive permeability of cell membranes. These processes are ultrastructurally reflected by, among others, appearance of intercellular spaces and basal infolded channels of the epithelial cells. Application of stereological measurements for their evaluation under various conditions of reabsorption would enable more complete insight into the passive transport processes.

The aim of our studies was to elaborate a method for evaluation of ultrastructural markers of active and passive transport in the renal tubules using a model of experimental diabetes insipidus.
Materials and methods

The studies were performed on 15 Wistar rats of both sexes, weighing 200–250g. The animals were divided into three groups each of five rats:

- Group I experimental diabetes insipidus,
- Group II control,
- Group III sham-operated rats.

Diabetes insipidus was induced by bilateral destruction of the supraoptic nuclei of the hypothalamus with high-frequency electric current (2MHz for 15 sec), using coordinates from the stereotactic atlas for rats [2].

After placing the rats in metabolic cages following the operation, 24-hour urine output, urine osmolality and urinary sodium and potassium concentrations were measured. After 12 days the animals were sacrificed and material for electron microscopic studies taken. In all groups the cortical layer of the kidney was cut into sections 1mm thick and then fixed in 5% glutaraldehyde with 2% paraformaldehyde in 0.1M cacodylate buffer, ph 7.4, at 0–4°C for three hours. All specimens were embedded in Araldite and then ultra thin sections were obtained by means of LKB III ultramicrotome and stained with uranyl acetate and lead citrate for examination in Philips EM-300 electron microscope.

Morphometric procedure was based on the techniques of Weibel [3,4]. In order to study the relationship between the mitochondrial compartments and the internal membrane, we introduced the following partition coefficients (E):

\[
E_{mm} = \frac{V_{mit}}{S_{im}} \times V_{mat} \quad ; \quad E_{ocm} = \frac{V_{mit}}{S_{im}} \times V_{oc},
\]

which express the volume of the matrix \((E_{mm})\) and external compartment \((E_{ocm})\), respectively, per unit of surface area of the internal mitochondrial membrane [5].

All calculations, mean values, standard error of the mean and coefficient of variation were performed on an SM 4A computer. All parameters were compared statistically by means of unpaired Student's 't' test and Cochrane-Cox and Whitney-Mann tests.

Results

In the rats with diabetes insipidus 24-hour urine output ranged from 27ml to 50ml, mean 37ml, while that in healthy and sham-operated animals was 9ml.

Stereological analysis of the proximal tubular epithelial cell mitochondria did not reveal any significant differences between diabetes insipidus and control groups. Also volume and surface area values of the intercellular spaces, as well as basal infolded channels, did not differ between the groups studied.

In the distal tubules significant differences were found between diabetes insipidus and control groups as regards the external membrane surface area and volume. Surface area was 9.7793\(\mu m^2/\mu m^3\) in the diabetes insipidus rats, while it was 8.0353\(\mu m^2/\mu m^3\) in the control groups. Relative volume of the external
membrane was 0.0588 per cent in the diabetes insipidus rats and 0.0387 per cent in the controls.

Statistically significant differences were also found in the mitochondrial internal membrane. Its surface area was 44.8155 μm²/μm³ in the diabetes insipidus animals, while in the controls it was 38.6785 μm²/μm³. Relative volumes of the internal membrane was 0.0882 per cent vs 0.0952 per cent, respectively.

Partition coefficient for the external compartment (E_0cm) significantly decreased from 0.0044 μm³/μm² in the controls to 0.0035 μm³/μm² in diabetes insipidus. Similarly, partition coefficient for the internal compartment lowered from 0.0209 μm³/μm² in the controls to 0.0199 μm³/μm² in diabetes insipidus (Figure 1).

![Figure 1. Mitochondria of distal tubule. Differences in configuration of mitochondrial membranes. a=control group; b=diabetes insipidus. Changes in direction to condensed steady state, × 27,000 (reduced for publication)](image)

Statistical analysis did not reveal any significant differences between the groups studied for the intercellular spaces and basal infolded channels within the proximal tubules.

However, in the distal tubules relative volumes of the intercellular spaces and basal infolded channels significantly decreased from 8.844 per cent in the controls to 6.1778 per cent in the diabetes insipidus rats.

In order to determine interrelationships between the intercellular space volume and surface area, the following coefficient was calculated:

734
\[ E_v = \frac{V_v}{S_v} \]

where \( V_v \) — relative volume of the intercellular space, \( S_v \) — relative surface area of the intercellular space. This coefficient is an exponent of the width of the intercellular space and basal infolded channels. In the rats with diabetes insipidus the value was 30 per cent less than in the control groups (Figure 2).

![Figure 2. The distal tubule. a=control group. Intercellular spaces and basal infolded channels are dilated. b=diabetes insipidus. Compared with control, narrow spaces are observed. ×10,400 (reduced for publication)](image)

Discussion

Our stereological data obtained from the analysis of the intercellular spaces and basal infolded channels were found to be consistent with the morphometric data reported by Pfaller [1].

An active ion transport as well as water reabsorption takes place mainly in the proximal tubule; our investigations on ultrastructural markers of ion and water transports did not reveal any differences between experimental diabetes insipidus and healthy or sham-operated animals. It confirms suggestions of other authors [6] who did not prove any effect of ADH on this nephron segment in rats.

Stereological analysis of mitochondrial ultrastructure enables determination
of their energy state [7,8], which may be reflected by changes from the condensed state through the transitional state to the orthodox one. Thus, energy states of the mitochondria may correlate with their function in active transport.

In our experiments the distal tubule mitochondria were found to be in the transitional state. In the course of diabetes insipidus their configuration was changing towards the condensed, i.e. low-energy, state. Thus, they seem to contribute to active transport to a lesser degree. In the diabetes insipidus rats 24-hour urinary sodium excretion was higher by 10.5 per cent than in controls. Assuming that sodium reabsorption in the proximal tubule was unaltered in all studied groups, it seems that the 10.5 per cent increase in the urinary sodium excretion results from its lowered reabsorption in the distal tubule.

In our studies volumes and surface areas of the intercellular spaces and basal infolded channels were taken as water transport exponents. Stereological analysis of these parameters did not reveal any significant differences in the proximal tubule between the groups studied. Thus water transport was also unaffected by ADH deficiency in this nephron segment.

However, a significant decrease in relative volumes of the intercellular spaces and basal infolded channels in the distal tubule is evidence for significant impairment of water transport. Assuming that approximately 20 per cent of water reabsorption takes place in the distal tubule, it constitutes approximately 180ml of primary urine. After subtraction of 9ml of final urine in the controls and 37ml in diabetes insipidus, we obtain 171ml and 143ml, respectively. These values theoretically define primary urine volume reabsorbed in the distal tubule. Thus, reabsorption in the distal tubule was reduced approximately 20 per cent in diabetes insipidus.

These calculations were performed under the assumption resulting from our studies that the proximal tubule function does not change in the course of diabetes insipidus. It is consistent with reports of other authors [6,9] and with observations that in rats vasopressin affects water reabsorption in the distal tubule [10].

If we compare results from our calculations for final urine with regard to 20 per cent of reabsorption in the distal tubule and 30 per cent reduction in the intercellular spaces and basal infolded channels, we think this difference results from reabsorption in the collecting tubules and the phenomenon of counter-current multiplication.

Results of our stereological studies in the mitochondrial metabolic states and volumes of the intercellular spaces and basal infolded channels indicate that they may be markers of ion transport and water reabsorption in the nephron.

References

7 Hackenbrock CR. J Cell Biol 1966; 30: 269
8 Hackenbrock CR. J Cell Biol 1968; 37: 345
10 Sasaki S, Imai M. Pflügers Arch 1980; 383: 215