Proximal convoluted tubules of the rats kidney - a stereological analysis

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Abstract

Background and Purpose: The aim of this work was to order quantitative parameters of the proximal convoluted tubules (PCT) in the normal rat kidney cortex. Volume density (V_V), both surface and specific surface density toward interstitium (S_{Vi} and S_{Vi}/V_V) and toward lumen (S_{Vl} and S_{Vl}/V_V) and thickness (T) of tubules epithelium have been stereologically ordered.
Material and Methods: Stereologically were analysed 170 test fields by lattice L_{36} on the paraffin sections of the three adult male Wistar rats kidney dyeing by PAS-method.
Results: The mean values of the variables analysed were: V_V=76.4% ±0.012; S_{Vi}=0.056µm-1 ±0.004; S_{Vl}=0.028µm-1 ±0.003; S_{Vi}/V_V=0.073µm-1 ±0.003; S_{Vl}/V_V=0.037µm-1 ±0.005; T=18,26µm ±0.897.
Conclusions: Stereological methods are making a very valuable contribution to science over recent years. We have used unbiased stereological counting methods to obtain objective quantitative parameters of the PCT epithelium in the normal rats' kidney cortex.

Key words: kidney, proximal convoluted tubule, stereology, rat.

Introduction

The nephron is the fundamental structural and functional unit of the kidney. Each nephron consists of a dilated portion, the renal, or malpighian, corpuscle; the proximal convoluted tubule (PCT); the thin and thick portions of the loop of Henle; and the distal convoluted tubule (9). The proximal thick segment of the nephron begins as the PCT at Bowman's capsule; it makes a short turn toward the cortex and then returns to its site of origin where it follows a very tortuous or convoluted course. It then enters a medullar ray and continues as the descending proximal straight tubule into the pyramid (21). PCT is the longest, most convoluted tubular part of the nephron (24) about 14 mm in length and 60µm wide in human ren (6). The PCT is lined by simple cuboidal epithelium. The cylindrical or pyramidal cell of this epithelium has a spherical nucleus and a strongly acidophilic granular cytoplasm. The cell apex, exposed to the lumen of the tubule, exhibits 1-1.3µm long abundant microvilli that form the so-called brush border. Because of the extensive interdigitations of the lateral membranes, no discrete cell margins can be observed with the light microscope between cells of the proximal tubule (9, 21).
Although all parts of the nephron, as well as the PCT, investigated and described morphologically in detail up today, our knowledge of renal quantitative parameters is still incomplete. The stereology as a method is mainly used for researches of glomeruli (1, 2, 7, 12, 13, 15, 23). Total numbers of glomeruli were estimated in the normal (4) and experimental (25) rat and in human (8) kidney. Usual techniques of stereological estimates of glomerular capillaries number and sizes were also used in researches of glomeruli in experimental conditions (3, 16). All studies that have used the technique of Nyengaard and Marcussen (18) to estimate the number of capillary loops contributed to the increase in total capillary length. These studies include studies of glomerular capillary growth in rats with lithium nephropathy (14), experimental diabetic nephropathy (19) and following unilateral nephrectomy (17). But very little has been published about quantitative parameters of the mayor proximal cortical nephron segments (11, 20, 22).
Knowledge of normal quantitative parameters of PCT epithelium is very important and might be useful for comparison based on objective numerical values obtained in investigations of different experimental and pathological conditions. The purpose of this work is based on the reasons mentioned above. Stereological analyses were performed on normally structured tubular part of rat's kidney cortex to obtain some main numerical parameters of PCT epithelium. The distal tubules were not evaluated owing to the difficulties in distinguishing with sufficient reliability between those structures and collecting ducts or vessels.

Material and methods

Rat kidneys used in this study were obtained from three adult male Wistar rats. After fixation in 10% neutral formaldehyde and embedding in paraffin, the 8µm-thick sections were stained by the periodic-acid-Schiff (PAS) reaction.
This study was performed by stereological analysis of 170 test fields of the ren tissue cortex. The sections for stereological analysis were used systematically and intermittently. Counting number was ordered from pilot measuring towards De-Hoff's (10) formula in which esti-

mated number "n" means number of test fields measuring needed for stereological analyse with 95% confidence.
Stereology as the methodology provides meaningful quantitative descriptions of the geometry of real three dimensional glob structures from measurements that are made on two dimensional images sampled from the glob (5). Stereological measuring was done on a light microscope by the semicircular multipurpose test system L_{36} after Mertz, at objective magnification x40. Referent space was the renal cortex and the proximal convoluted tubule epithelium was the investigated phase (Fig.1).
We calculated by means of standard stereological procedures volume density (V_V) of the PCT epithelium, its surface density (S_{Vl}) and specific surface density (S_{Vl}/V_V) toward lumen, its surface density (S_{Vi}) and specific surface density (S_{Vi}/V_V) toward interstitium and its thickness (T).
Statistical evaluation of the data included calculation of basic descriptive statistic parameters. We calculated mean value - σ, standard deviation - SD and standard error - SE of results obtained for each stereological variable.

Results

Results of rat kidney quantitative analyse are presented in Table 1.
The results of the stereological analyses have shown that volume density (V_V) of PCT epithelium varied from 0.750 to 0.774μm^o in three investigated rats kidneys. The average volume density (σ±1SE) was 0.764± 0.007μm^o.
Surface density (S_{Vl}) of PCT epithelium toward lumen, or luminal surface of epithelium, in three investigated rat's kidneys varied from 0.024 to 0.030μm-1. The average surface density (σ±1SE) was 0.028± 0.002μm^{-1}.
Surface density (S_{Vi}) of PCT epithelium toward interstitium, or basal surface of epithelium, varied from 0.052 to 0.059 μm^{-1} in our investigation, and the average value (σ±1SE) of this stereological variable was 0.056± 0.002μm^{-1}.
Specific surface density (S_{Vl}/V_V) of PCT epithelium luminal surface varied from 0.031 to 0.041μm^{-1}, and the average value (σ±1SE) was 0.037± 0.003μm^{-1}.
Specific surface density (S_{Vi}/V_V) of PCT epithelium toward interstitium varied from 0.070 to 0.076μm^{-1}, and the average value (σ±1SE) was 0.073± 0.002μm^{-1}.
Thickness (T) of PCT epithelium in three investigated rats kidneys varied from 17.440 to 19.218μm,and the average value (σ±1SE) was 18.261±0.518μm.

Discussion

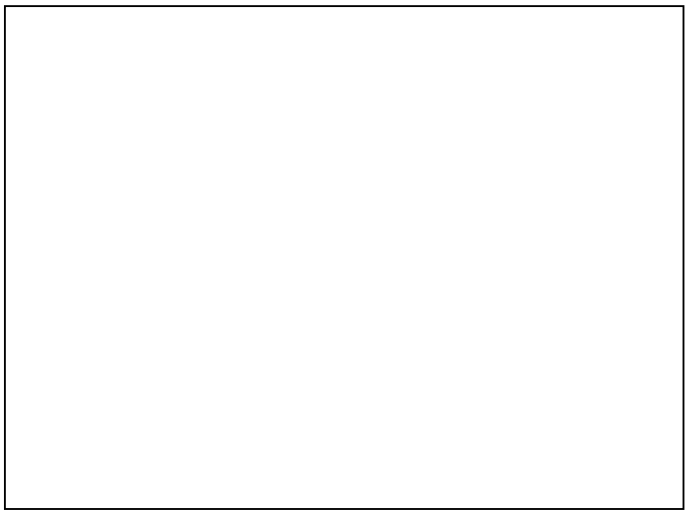
The present results indicate that the useful information can be obtained about rat kidney by stereological investigation. The only requirement was to recognise and distinguish the proximal convoluted tubules epithelium from the other structures in the renal cortex. Sections in our investigation were stained by the periodic-acid-Schiff (PAS) reaction because previous morphologic descriptions (9) pointed at a well-developed, PAS-positive basement membrane around the proximal convoluted tubule and at its microvillar brush border lined by a PAS-positive cell coat (a structure rich in glycoproteins). We have described the estimation of volume density, surface density, specific surface density and thickness as an example of using of stereological methods.
Volume density, as a relative stereological variable, denoting how much of the volume is taken up by researched phase. The results of our stereological analyse have shown that the volume part of proximal convoluted tubules epithelium was 76.4% of the rats renal cortex.
Surface density, as a relative stereological variable, denoting how much space a certain surface had taken up when compared to the volume unit. In our investigation the average surface density of luminal surface of proximal convoluted tubules epithelium (S_{Vl}) was 0.028± 0.002μm^{-1} and the average value of its surface toward interstitium (S_{Vi}) was much greater (0.056± 0.002μm^{-1}).
Specific surface density denotes how a certain surface changes in comparison to its volume. Specific surface density (S_{Vl}/V_V) of proximal convoluted tubules epithelium luminal surface was 0.037± 0.003μm^{-1} and the specific surface density of proximal convoluted tubules epithelium toward interstitium (S_{Vi}/V_V) was much greater and that 0.073± 0.002μm^{-1}.
The mean thickness of 18.261μm of PCT epithelium

Table 1 The main morphometric parameters of epithelium of the rat renal proximal convoluted tubules

Kidney	VV	SVi	SVl	SVi/ VV	SVl/ VV	T
1.	0.774	0.059	0.030	0.076	0.038	17.440
2.	0.750	0.052	0.030	0.070	0.041	18.124
3.	0.767	0.056	0.024	0.073	0.031	19.218
σ	0.764	0.056	0.028	0.073	0.037	18.261
SD	0.012	0.004	0.003	0.003	0.005	0.897
SE	0.007	0.002	0.002	0.002	0.003	0.518

mean value - σ, standard deviation - SD, standard error - SE

Figure 1 Proximal convoluted tubule (H) in the normal rat' kidney cortex (stained by PAS method, x180)



obtained in our study is compatible with observation that the thin part of the loop of Henle, which is a continuation of the proximal convoluted tubule, has an external diameter of about 12µm, the smaller collecting tubules have a diameter of approximately 40µm and the diameter of the collecting ducts reaches 200µm near the papillae (9) and with observation that proximal convoluted tubule is about 60µm wide in human ren (6). Our findings are also compatible with previous findings of Kazimierezak et al. (11). They measured diameters of the cortical proximal and distal tubules of the unilaterally nephrectomized and the control male, four weeks old, Wistar rats by an eyepiece with inserted scale. They have indicated a progressive increase in diameter of the cortical tubules between the second and the twenty-eighth days of experiment. The diameter of PCT increased from 32µm to 42µm. The mean thickness of 18.261µm of PCT epithelium obtained in our study indirectly corroborates their findings of PCT diameter because their experimental animals were much younger than adult animals in our investigation.

Despite differences in the stereological techniques and animal age, sex and weight, our results are quantitatively very similar to those of Seyer-Hansen et al. (22) and Pfaller et al. (20) and indirectly corroborate theirs. Seyer-Hansen et al. (22) studied different rats' renal structures during the initial phases of compensatory renal hypertrophy. Their measurements were made both on intact and on nephrectomized animals. They estimated volume fractions of proximal tubular cells and lumina with cortex as the reference volume, the average luminal and external diameter of the proximal tubulus and average proximal tubulus cell height as half the difference between the two diameters. In intact control animals tubule cell volume was 345±14mm^3, luminal diameter was 19.2±2µm and epithelial cell height was 10.2±1µm. Pfaller et al. (20) studied the compensatory hypertrophy in different renal cortical structures in rats 10 and 21 days after unilateral nephrectomy and in sham controls by quantitative morphological/stereological analysis and found the PCT responds with "radial" hypertrophy (thickening of the tubular epithelial wall), while the DCT undergoes "length" hypertrophy (increase of tubular length without thickening of the tubular wall and without an increase in number of cells). Absolute volume of epithelial PCT compartments contained in the renal cortex was about 310ml. Absolute surface areas of the luminal (3.4m^2) and basolateral (2.3m^2) PCT cell membrane domains contained in the renal cortex were determined in sham controls. The enlargement in PCT epithelial volume is accompanied by changes in the total luminal and basolateral membrane surface areas of cortical tubule segments. Although they didn't directly measure, they conclude an increase in the thickness of the PCT epithelial cell layer. In conclusion, the present findings have confirmed previous morphologic findings, identified some new and important correlations, and suggested some correlations that may emerge as statistically significant once additional kidneys have been analysed. Meanwhile, the knowing of normal quantitative parameters of proximal convoluted tubules epithelium is important for extending our total knowledge about ren.

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