Study of Morphometric Parameters of Podocyte Foot Processes in Impaired Calcium Balance

V. A. Titova, I. N. Pavlenko, A. Yu. Balakina, V. V. Barabanova, and S. A. Zueva

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Study of morphometric parameters of podocyte foot processes showed that the increase of parathyroid hormone blood level causes dilatation of podocyte foot processes resulting from accumulation of calcium in the cells. Influence of the hormone on filtration processes in glomeruli as a result of its effect on podocytes is discussed.

Key words: *parathyroid hormone; chronic renal failure; podocytes*

The development of chronic renal failure (CRF) is associated with disturbances in calcium homeostasis in the organism that causes irreversible pathological alterations in the kidney and other organs [3]. One of the main causes of impaired calcium balance in CRF is an increased blood level of parathyroid hormone (PTH) resulting from its reduced catabolism and elimination [10]. PTH regulates filtration processes in the glomerular filter [7] through PTH receptors on glomerular cells [11]. However, the mechanism of the effect of this hormone on glomerular filter, in particular on the function of visceral renal epithelium (podocytes), is not clear. Podocytes possess considerable cellular mobility due to reactions of their cytoskeleton proteins [1], they can change the shape and the size of foot processes in response to physiological stress or disease [5]. Therefore, we use morphometric parameters (MP) of foot processes for evaluating the effect of PTH on podocytes in both exogenous and endogenous increase in the blood level of the hormone, i.e. in experimental CRF and experimental hyperparathyroidism.

MATERIALS AND METHODS

Experiments were carried out on male Wistar rats weighing 200 g. CRF was modeled by subtotal nephrectomy; the animals were sacrificed 3 months postoperation [8]. The content of calcium ions was significantly decreased (0.85±0.0018 *vs* 1.18±0.004 mmol/liter in the control, $p<0.001$). Experimental hyperparathyroidism was induced by administration of exogenous PTH in a dose of 50 units for 14 days. The treatment considerably reduced the content of calcium ions in experimental animals (0.799±0.011 *vs* 1.18±0.004 mmol/liter in the control, $p<0.001$). Podocyte ultrastructure was studied using conventional schedule of tissue processing [4]; cell location of calcium ions was determined by a pyroantimonate method [9]. In addition to long-term effect of exogenous PTH, a reaction of foot processes to a single injection of PTH in the same dose was studied 2 and 24 hours postinjection. As an alternative to the effect of PTH or CRF, intact renal tissue was treated with EGTA, a specific chelator of calcium ions. Tissue fragments were treated with 1.12 M phosphate buffer, containing 7% sucrose, and 3 mM of EGTA for 1 or 5 min. Control samples of the same size were incubated for 1 and 5 min in a buffer without EGTA [14].

Ultrathin sections were prepared using an Ultracut ultratome and examined under Hitachi-300 electrone microscope (×30,000). A method to study dendrite spike MP was used, because of similarity between foot processes and dendrite spikes in size and configuration [4]. The following MP were compared: minimum and maximum diameters of foot process, the length of the contact between foot process and basement membrane,

Laboratory of Kidney Pathophysiology and Pathological Morphology, Institute of Nephrology, I. P. Pavlov Medical University, St. Petersburg; Laboratory of Cell Physiology and Molecular Immunology, A. A. Ukhtomskii Institute of Physiology, St. Petersburg State University

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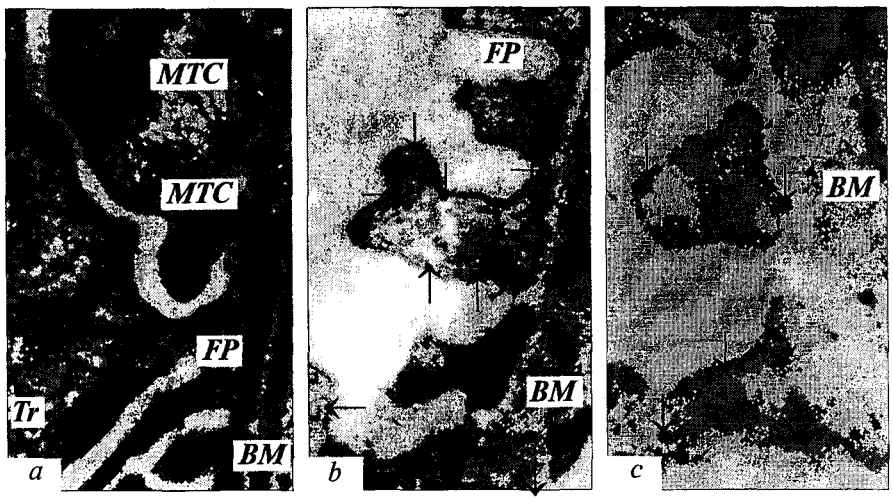

Fig. 1. Electron micrograph of podocytes in experiments with administration of exogenous parathyroid hormone. *a*) lengthening of foot process contact with the basement membrane; *b*, *c*) calcium pyroantimonate precipitates (arrows) in podocyte (*c* — histological appearance without contrast). Tr: trabecula, FP: foot process; BM: basement membrane, MTC: mitochondria. Magnification: *a* — ×10,000; *b* — ×20,000; *c* — ×40,000.

and the length and perimeter of the foot process. Each parameter was measured in at least 60 foot processes; the results are presented as histograms. The data were compared using the Kolmogorov's test [2].

RESULTS

Under standard conditions foot processes had dense matrix, predominantly located near their contact with the basement membrane. Cisternae of the endoplasmic reticulum were seen at the base of foot process; in the cytoplasm, grouped polyribosomes, solitary clathrin vesicles, and microfilament network were seen in foot process and at the site of its orifice from a trabecula. Analysis of electron micrographs, obtained 3 months after subtotal nephrectomy showed clearing of podocyte matrix. Isolated microtubules and rarefied microfilament network were seen in the cell body cytoplasm; multivesicular bodies, clusters of mitochondria, and cisternae of the endoplasmic reticulum were found at the base of foot process. Multiple vesicles were seen in the cell body and in foot processes.

Single and long-term administration of PTH caused different ultrastructural changes in podocytes. Two hours after injection of the hormone, matrix of the foot processes was condensed. Microtubules and microfilament network were clearly seen in the cytoplasm of trabeculae. Free and membrane-incorporated clathrin vesicles were often found at the base of foot process. Free ribosomes or polyribosomes, as well as multivesicular bodies, isolated mitochondria, and vesicles were found in trabeculae.

The same ultrastructural picture was observed 24 h after PTH injection. Unlike single PTH injection administration of PTH for 14 days induced pronounced rarefaction of filamentous matrix in foot processes and cell body. In addition, isolated microtubules were seen in the cytoplasm of trabeculae (Fig. 1, *a*).

Incubation with EGTA induced condensation of the matrix in the foot processes and appearance of microtubule bundles and compact microfilament network in trabeculae. Cytochemical study of calcium distribution after 14-day PTH administration using a pyroantimonate technique revealed a considerable enlargement of calcium phosphate granules, compared to standard experimental conditions. Multiple electron-dense granules were found in mitochondria, cisternae of the endoplasmic reticulum, Golgi apparatus, and on

TABLE 1. Changes in Ultrastructural Morphometric Parameters of Podocyte Foot Processes under Different Experimental Conditions

Treatment	Length of foot process	Minimum diameter	Length of contact with basement membrane	Maximum diameter	Perimeter
PTH, 2h	↑	↓	—	—	↑
24 h	↑	—	↑	—	↑*
14 days	—	↑	↑*	↑	↑*
EGTA	↑	↓	↓	↓	↓*
CRF	↑	↑	↑*	↑*	↑*

Note: Arrow downwards: decrease; arrow upwards: increase; dash: no changes; *$p<0.05$ compared to the control.

the external and internal surface of the foot process membrane, as well as along its contact with the basement membrane (Fig. 1, *b* and *c*).

Histogram of MP distribution showed lengthening of foot process contact with the basement membrane in CRF (Table 1, Fig. 2, *d*). Two and 24 hours after PTH injection, no significant changes were noted, but 14-day PTH administration significantly increased this parameter (Table 1, Fig. 2, *b*). Exposure to EGTA induced alterations, opposite to the effect of PTH (insignificant) (Table 1).

A significant deviation of MP from normal after prolonged administration of PTH, as well as lower density of filamentous matrix can be associated with PTH-dependent accumulation of Ca^{2+} in the cytoplasm of podocytes that affects the integration of cytoskeletal structures. The shifts in MP of foot processes caused by experimental CRF and PTH were similar, i. e. these factors produced analogous changes in the cytoskeleton. The most significant MP is the length of foot process contact with the basement membrane (Table 1, Fig. 2). The increase in this parameter reflects dilatation of foot processes (as the beginning of deformation), and their fusion associated with proteinuria [5]. In our experiments, PTH blood level in animals with CRF 2- to 3-fold surpassed the normal value, which probably underlied the observed partial disintegration of the cytoskeleton.

When analyzing MP of podocytes after a single PTH injection, we found no accumulation of Ca^{2+} in the cell (Table 1) probably due to insufficient Ca^{2+} concentration or not completely exhausted Ca^{2+}-accumulating capacity of cell depots.

In experimental series with administration of exogenous PTH, pyroantimonate precipitation assay revealed Ca^{2+} accumulation in cell depots: mitochondria, Golgi apparatus, endoplasmic reticulum, as well as in the near-membrane layers (predominantly intracellular) along the perimeter of foot process and within its contact with the capillary basement membrane (Fig.

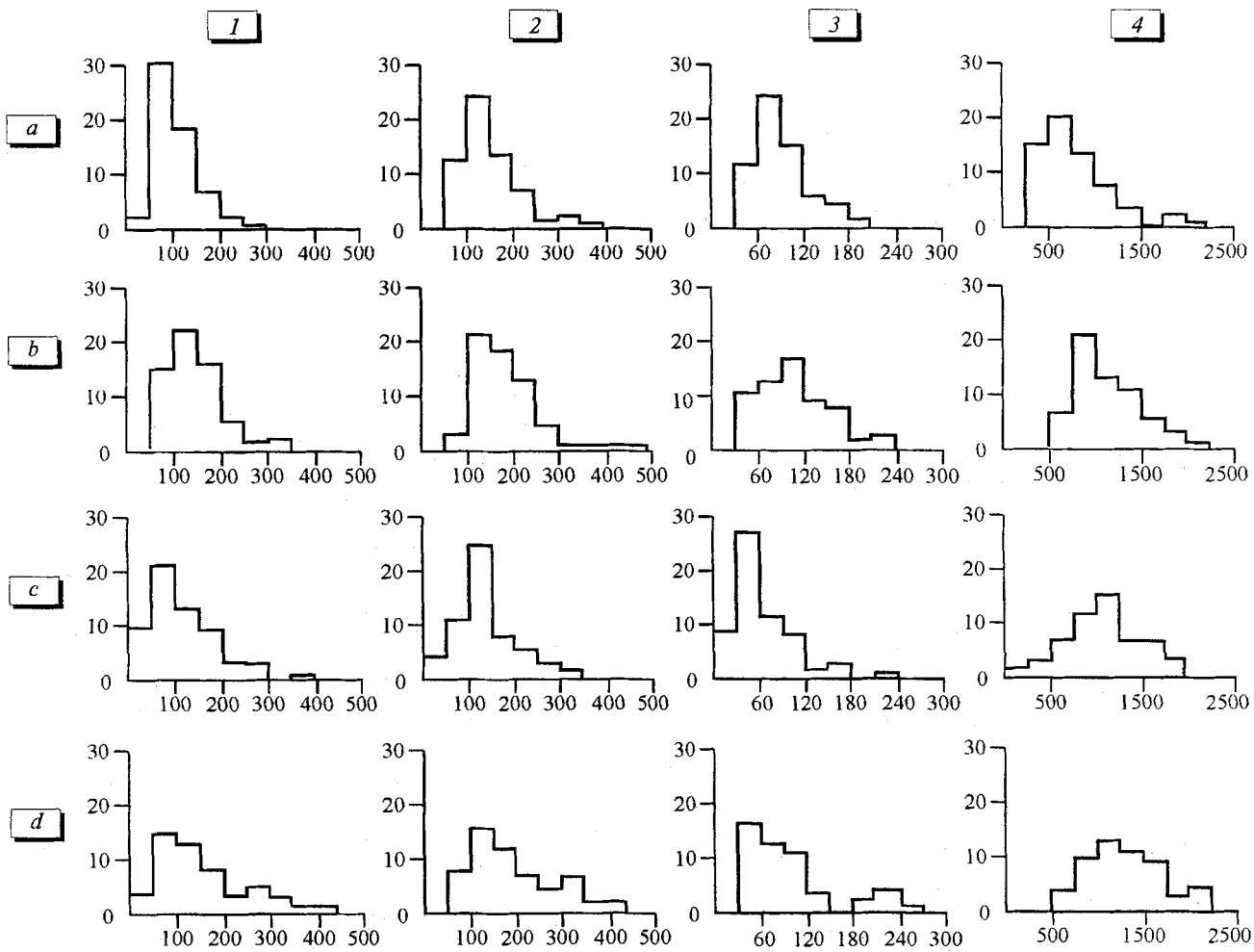

Fig. 2. Histograms of distribution of morphometric parameters of podocyte foot processes. Ordinate: number of foot processes; abscissa: value, nm. *a*) control; *b*) 14-day administration of parathyroid hormone; *c*) EGTA; *d*) chronic renal failure. *1*) length of foot process contact with the basement membrane; maximum (*2*) and minimum (*3*) diameters of foot process; 4) perimeter.

1, *b, c*). This can be due to excessive accumulation of Ca^{2+} in the cell exceeding the capacity of intracellular depots and impaired Ca^{2+} influx and efflux. Ca^{2+} accumulation in the glycocalix is an evidence of its deposition in anionic sites that reduces the negative surface charge of foot processes.

Additionally, the effect of an alternative treatment, i. e. incubation in a calcium-free medium containing 3 mM of EGTA, on MP of podocytes was studied. If reorganization of the cytoskeleton is associated with PTH-dependent accumulation of Ca^{2+} in cells, the incubation in a calcium-free medium will induce opposite shifts in MP. The observed qualitative alterations (an increased number of microtubules in the cytoplasm of foot process) confirmed our assumption. Morphometric analysis revealed no significant changes in MP; however, we observed a distinct trend towards shortening of the contact between foot process and the basement membrane in contrast to its lengthening caused by PTH (Table 1).

Thus, our findings suggest that dilatation and deformity of foot processes are associated with changes in intracellular Ca^{2+} content. PTH can trigger reorganization of the cytoskeleton, since its accumulation in the blood increased Ca^{2+} level in foot process and decreased the negative charge on its surface [12]. Therefore, in addition to the well-known regulation of glomerular filtration via mesangial cells [8], PTH can modulate this function via the reaction of podocytes [6]; purposeful correction of the intracellular Ca^{2+} level can restore podocyte surface charge and function.

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