Excretion of Proteins by Rat Kidney during Various Types of Diuresis

A. V. Kutina, V. V. Zakharov*, and Y. V. Natochin

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 146, No. 12, pp. 613-616, December, 2008
Original article submitted July 22, 2008

Experiments on healthy rats showed that increased diuresis induced by administration of water, polyethylene glycol 400, furosemide, or 1-desamino-arginine-vasotocin is associated with increased protein excretion by the kidneys. The results can be explained by enhanced filtration of plasma proteins in glomeruli during polyuria of various geneses.

Key Words: *protein excretion; urinary protein electrophoresis; saluresis; water diuresis; osmotic diuresis*

The initial stage of urine production is fluid ultrafiltration in glomeruli based on separation of water and dissolved low-molecular-weight substances from proteins to retain the latter in blood plasma. The proteins are detected in the urine under pathological conditions and during intensive physical exercise [5]. Proteinuria attests to unfavorable state of the kidneys and is manifested as microalbuminuria or loss of proteins up to 50 g/day [4,9]. Normally, negligible amount of proteins is filtered in the glomeruli and virtually completely reabsorbed in the proximal segments of the nephrons [8]. Under pathological conditions, some proteins (such as Tamm—Horsfall protein) can be excreted by epithelium of renal tubules [12].

This study focused on the problem whether kidneys of healthy animals kept under normal conditions can excrete proteins of tubular (not glomerular) origin and whether drastic increase in diuresis affects protein excretion. To answer these questions, we examined excretion of proteins by the kidneys at various types of diuresis, when increase in urine production depended on inhibition of reabsorption in proximal and distal segments of the nephron or in the collecting tubules.

MATERIALS AND METHODS

Experiments were carried out on female Wistar rats weighing 150-220 g. Five series of experiments were performed: intragastric administration of 5 ml water (via a tube) into the stomach of non-narcotized rats (series 1); intramuscular injection of 0.1 ml 1% furosemide (series 2); administration of 0.4 g 25% glycerol into the stomach via a tube (series 3); intravenous infusion of 0.75 ml 40% polyethylene glycol 400 (PEG-400) under nembutal narcosis (0.75% nembutal+0.37% chloralose, 0.6 ml) (series 4); intramuscular injection of 0.05 nmol 1-desamino-arginine-vasotocin (1-dAVT, series 5). Intact rats comprised the control group. The doses of the injected chemicals are given per 100 g body weight.

The rats were placed into tight boxes with wire floor and conical narrowing. The urine was collected into a test tube over 2 h. We measured the volume of urine, creatinine concentration measured (kinetic method using Jaffe reaction), total protein measured with pyrogallol reactive on an EOS Bravo W automatic biochemical analyzer.

Urine samples were diluted with water to protein concentration of 0.1 mg/ml. Acetone (3 ml) was added to each urine sample (0.5 ml), and the

I. M. Sechenov Institute of Evolution Physiology and Biochemistry, Russian Academy of Sciences, St. Petersburg; *B. P. Konstantinov St. Petersburg Institute of Nuclear Physics, Russian Academy of Sciences, Gatchina, Russia. ***Address for correspondence:*** natochin@iephb.ru. Y. V. Natochin

0007-4888/08/1466-0671

proteins were precipitated overnight at -20°C. Then the samples were centrifuged at 12,000*g* for 20 min at -20°C, the precipitate was dried at room temperature, and dissolved in buffer for electrophoresis (0.0625 M tris-HCl, pH 6.8, 2% dodecyl sulphate, 10% glycerol, 10 mM dithiothreitol). Electrophoresis under denaturating conditions was performed by the standard method [10] in 12% PAAG. The gels were stained with colloid solution of Coomassie G250. Vasotocin analog 1-dAVT was synthesized by Sintez Peptidov Company [2].

The results were analyzed statistically using Statistica 6.0 software. Significance was assessed at $p<0.05$ with Kruskal—Wallis test and Dann test for multiple comparison [1].

The study protocol was approved by Ethical Committee of I. M. Sechenov Institute of Evolution Physiology and Biochemistry, Russian Academy of Sciences.

RESULTS

Administration of 5% water load increased water diuresis by 11.7 times in comparison with the control (Table 1). Intramuscular injection of furosemide or 1-dAVT increased diuresis by 11.7 and 4.3 times, respectively (Table 1). Administration of glycerol or PEG-400 resulted in osmotic diuresis with a 4-5-fold increase in urine production. The use of PEG-400, a liquid polymer, as an osmotic diuretic agent stimulated diuresis without administration of large volumes of fluid [3] necessary during infusion of mannitol solution. Glycerol administered *per os* induced osmotic diuresis in non-narcotized rats. Injection of 1-dAVT dramatically decreased sodium reabsorption, which increased natriuresis and diuresis and enhanced reabsorption of osmotically free water [2].

All types of experimental diuresis were characterized by enhanced protein excretion (by 200-300% compared to control, Table 1). Surprisingly, each type of diuresis was mediated by different mechanisms and involved different tubular segments: 1) water diuresis depended on inhibition of vasopressin secretion and decrease in water permeability of the collecting tubules; 2) osmotic diuresis resulted from inhibition of fluid reabsorption in the proximal tubules due to the presence of non-reabsorbing osmotically active substances in this segment (in this study, PEG-400 or glycerol) [3,5]; 3) furosemide blocked $Na^+,K^+,2Cl^-$-cotransporter in the luminal membrane of cells in the thick ascending limb of the loop of Henle [5]; and 4) 1-dAVT inhibited sodium reabsorption in distal segment of nephron [2].

TABLE 1. Urine Output and Protein Excretion by Rat Kidneys during Various Types of Diuresis over 2 h after the Start of the Experiment (*M*±*s*)

Experimental conditions	Urine volume, ml	Protein excretion, μg
Control, *n*=9	0.3±0.2	130±34
Glycerol, *n*=5	1.3±0.4	261±36
PEG-400, *n*=9	1.6±0.4*	331±81**
Furosemide, *n*=9	3.5±0.4**	377±80**
1-dAVT, *n*=9	1.3±0.2**	358±50**
Water load, *n*=9	3.5±0.7*	349±168**

Note. *$p<0.05$, **$p<0.01$ compared to the control.

Enhancement of protein excretion in various types of diuresis (independent on the location of factors promoting polyuria) could be determined by two phenomena: either excretion of various proteins in various subdivisions of the nephron, or elevation of protein filtration in the renal glomeruli. The first possibility could result from various degree of reabsorption of filtered proteins in proximal tubular segment and secretion of some proteins (such as Tamm-Horsfall protein) in other nephron subdivisions [12]. To answer the question whether the excreted proteins are different in various types of diuresis we performed electrophoresis of urinary

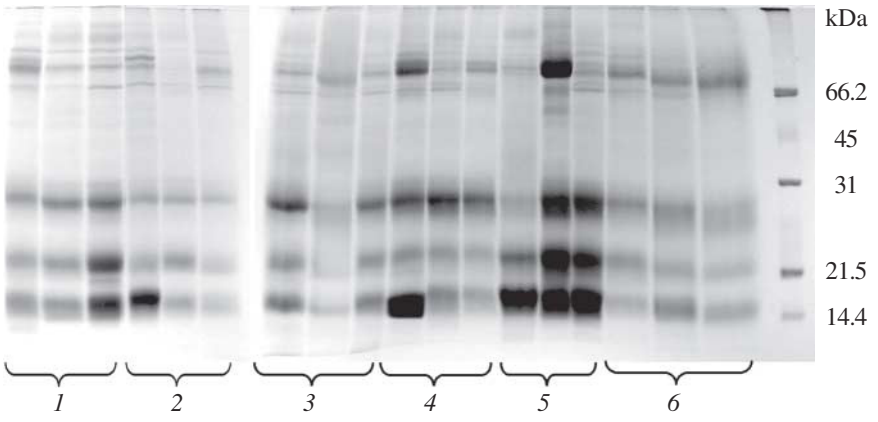

Fig. 1. Electrophoresis of urinary samples. *1*) water load; *2*) furosemide; *3*) glycerol; *4*) PEG-400; *5*) control; *6*) 1-dAVT.

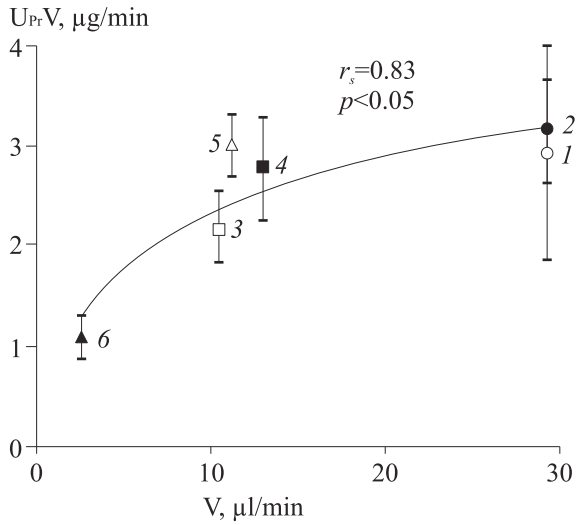

Fig. 2. Protein excretion as a function of diuresis. *1*) water load; *2*) furosemide; *3*) glycerol; *4*) PEG-400; *5*) 1-dAVT; *6*) control. Data are presented as the mean and the scatter within 95% confidence interval. r_s is Spearmen rank correlation coefficient.

proteins. In all experiments, the major detected substances were low-molecular-weight proteins and albumin. The spectrum analysis revealed no pronounced differences between the excreted proteins (Fig. 1).

The second possibility can be realized in different effect of the above factors on glomerular filtration. Since excretion of the proteins significantly increased with increasing the rate of urine production, these processes were compared and a clear-cut correlation between the rate of diuresis and protein excretion was revealed (Fig. 2). Since excretion of a protein filtered in glomeruli depends on its reabsorption, which can be different for various proteins, we calculated the rate of protein excretion in relation to glomerular filtration. Such calculations are necessary, because administration of water increases the volume of extracellular fluid, which promotes the increase in the rate of glomerular filtration [11]. Injection of furosemide [13] and infusion of PEG-400 [3] elevated renal blood flow and the rate of glomerular filtration. Another mechanism probably mediated the effect of vasotocin analog on the glomerular apparatus. Both vasotocin and vasopressin modulate vascular tone and water reabsorption via stimulation of V_1 and V_2 receptors, respectively [6]. Different effects produced by pituitary nonapeptides on the tone of afferent and efferent arterioles [7] can modulate intraglomerular pressure and conditions of protein filtration into the capsule lumen. Taken together, these data suggest that during administration of the test substances, the increase in the rate of urine production was accompanied by simultaneous effect on the rate of glomerular filtration and/or on the state of glomerular filter for proteins. Both changes could be reflected in elevation of protein excretion per milliliter of fluid that had been filtered in the glomeruli. Indeed, excretion of protein per milliliter of glomerular filtrate increased in any type of diuresis (Fig. 3). There was a strong positive correlation between protein/creatinine excretion rate and urine production (r=0.97, p<0.05).

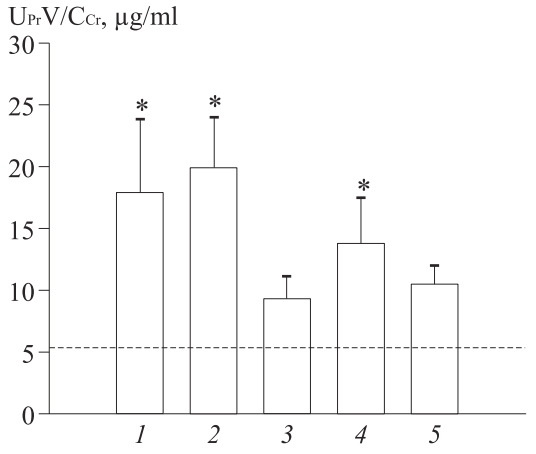

Fig. 3. Excretion of protein by rat kidneys in relation to the rate of glomerular filtration. *1*) water load; *2*) furosemide; *3*) glycerol; *4*) PEG-400; *5*) 1-dAVT. Dashed line shows the control level. The data are presented as the mean and the scatter within 95% confidence interval. **p*<0.05 compared to the control value.

Thus, our study showed that increase in the urine output in healthy animals can be accompanied by elevation of protein excretion in the kidneys. Moreover, protein excretion per milliliter of the glomerular filtrate increased, which indicates primary or secondary involvement of the glomerular apparatus into protein filtration during polyuria caused by the action of physiologically active substances on the tubular cells. These data make it possible to reconsider the role of the intrarenal system of the glomerular-tubular balance. These data are also interesting for clinical physiology to assess the development of proteinuria and to examine the role of microalbuminuria in some pathological processes.

The study was supported by the Russian Foundation for Basic Research (grant No. 08-04-00610), by Leading Scientific Schools Grant of President of Russia (grant No. NSh-4414.2008.4), and by Fundamental Study Program OBN of the Russian Academy of Sciences.

REFERENCES

1. S. Glants, *Medical and Biological Statistics* [in Russian], Moscow (1999).

2. Y. V. Natochin, T. A. Kanashkina, E. I. Shakhmatova, *et al*., *Eksp. Klin. Farmakol*., **71**, No. 2, 32-35 (2008).
3. Y. V. Natochin and E. I. Shakhmatova, *Byull. Eksp. Biol. Med.,* **69**, No. 4, 79-81 (1970).
4. V. N. Khirmanov, *Ter. Arkh.*, **76**, No. 10, 78-84 (2004).
5. D. A. Sheiman, *Kidney Pathophysiology* [in Russian], St. Petersburg (1999).
6. L. Bankir, *Cardiovasc. Res*., **51**, No. 3, 372-390 (2001).
7. N. Bouby, M. Ahloulay, E. Nsegbe, *et al., J. Am. Soc. Nephrol*., **7**, No. 6, 842-851 (1996).
8. E. I. Christensen and J. Gburek, *Pediatr. Nephrol*., **19**, No. 7, 714-721 (2004).
9. G. D'Amico and C. Bazzi, *Kidney Int*., **63**, No. 3, 809-825 (2003).
10. U. K. Laemmli, *Nature*, **227**, 680-685 (1970).
11. A. B. Roald and O. Tenstad, *Acta Physiol. Scand*., **182**, No. 2, 197-204 (2004).
12. M. D. Säemann, T. Weichhart, W. H. Hörl, and G. J. Zlabinger, *Eur. J. Clin. Invest*., **35**, No. 4, 227-235 (2005).
13. L. L. Woods, D. R. De Young, and B. E. Smith, *Am. J. Physiol*., **261**, No. 5, Pt. 2, F815-F823 (1991).