Pflügers Arch. 324, 279—287 (1971)
© by Springer-Verlag 1971

Handling of Uric Acid by the Rat Kidney

I. Microanalysis of Uric Acid in Proximal Tubular Fluid *

R. Gregger, F. Lang, and P. Deetjen

Institute of Physiology, University of München

Received November 17, 1970

Summary. Uric acid transport in the rat proximal tubule was studied by micropuncture and a new method of chemical ultramicro analysis. Under normal free-flow conditions at physiological levels of uric acid plasma concentrations a net secretion of uric acid in the proximal tubule was demonstrated. In these experiments the clearance ratio of uric acid to inulin was in the range of 0.4 which is normal in antidiuretic rats. Net reabsorption of uric acid, therefore, took place in the kidney, but certainly not in the proximal tubule as previously suggested.

Key-Words: Uric Acid Secretion — Micropuncture — Renal Tubule — Ultramicro Analysis.

Zusammenfassung. Mit Hilfe der Mikropunktionstechnik und einer neu entwickelten Methode der Ultramikroanalyse wurde der Harnsäuretransport am proximalen Tubulus der Rattenniere untersucht. Unter normalen Bedingungen ohne Harnsäureinfusion und ohne osmotische Diurese konnte eine Harnsäure-Netto-Sekretion im proximalen Tubulus nachgewiesen werden. Dabei war in diesen Experimenten die Inulinclearance etwa doppelt so groß wie die Harnsäureclearance. In der Bilanz wurde also mehr Harnsäure resorbiert als sezerniert, aber nicht, wie früher angenommen, im proximalen Tubulus.

Schlüsselwörter: Harnsäuresekretion — Mikropunktion — Nierentubulus — Ultramikroanalyse.

The characteristics of uric acid transport in the kidney have been studied by clearance and stop flow techniques. From these experiments it was concluded that uric acid is reabsorbed in the proximal tubule and secreted in the distal convolution [3, 9, 18, 20]. However, this concept was recently put into question by the observation that the distal tubule is almost impermeable to uric acid [12, 15, 16, 22]. On the other hand, it was shown that under certain circumstances uric acid clearance is suppressed by certain weak organic acids like PAH [21]. This finding suggests that uric acid may use the same mechanism of organic acid transport by which PAH and other organic acids are secreted in the proximal tubule (v. 4). In order to examine the handling of uric acid

* This study was supported by a grant of the Deutsche Forschungsgemeinschaft.

in the proximal tubule with a direct approach, we measured in micropuncture experiments the concentration of uric acid along the proximal tubule during free flow in the rat kidney.

Methods

General Procedures. We used 200—300 g Wistar rats anaesthetized with Inactin (120 mg/kg body weight i. p.). An external jugular vein was catheterized for infusion of Ringer's solution (containing 2 g/100 ml inulin) at a rate of 0.1 ml/kg min. A femoral catheter permitted sampling of arterial blood. To determine urine concentrations of inulin and uric acid, bladder urine was sampled through a catheter.

The left kidney was exposed and immobilized in a plexiglass cup. Preheated mineral oil was continuously perfused over the exposed surface of the kidney to maintain it at body temperature. During the entire experiment body temperature was maintained constant at $37.5 \pm 0.2°$ C by feed back regulated heating of the operating table using a rectal NTC probe and an electronic amplifier.
We only used rats which after intravenous dye injection had a proximal passage time of less than 12 sec and a distal reappearance time of less than 35 sec.

We punctured with glass capillary pipettes (outer tip diameter 10—12 μ) filled with castor oil. Collection time was 5—10 min and never exceeded 15 min. Puncture sites were injected with coloured Neopren solution. After macerating the kidney in HCl, the Neopren cast was dissected out and the puncture site identified to determine its distance from the glomerulus.

Chemical Analysis. Inulin concentration was measured in blood and urine by the Anthron method [6] and in tubular fluid by a micromodification of this method [11]. The use of a new designed ultramicrocuvette with a filling volume of less than 10^{-3} ml [17] permitted the reduction of the tubular sample volume to about 2—3 nanoliters.

Uric acid in blood, urine and standards was analysed by an uricase method (Boehringer-Mannheim Test Combination).

At normal plasma levels uric acid concentrations in the tubular fluid is rather low in rats, ranging from 0.1—0.3 mMol/l. Since the standard chemical methods described in the literature result in low extinction coefficients (uricase methods about 8.0 l/mMol × cm, uricase-peroxidase methods 13.5 l/mMol × cm [14]), it is difficult to perform quantitative analysis when one is limited by a maximum sample size obtainable with collection periods no longer than 10 min (approximately 50 nanoliters). We now have modified the phosphotungstic acid method and adapted it to ultramicro dimensions and we are now able to analyze uric acid in quantities as low as 10^{-12} mols. The determination involves the reduction of a certain complex of phosphotungstic acid by urate [5]. Previous authors used carbonate to buffer the final mixture to an alkaline pH [2]. Working with this method we sometimes got a cloudy precipitation and, therefore, we substituted borate for sodium carbonate.

Reagents. Phosphotungstic acid: 10 g of sodium tungstate ($Na_2WO_4 \cdot 2\,H_2O$) are dissolved in 80 ml of water. 8.0 ml of 85% phosphoric acid are added. This solution is boiled gently in a water bath for 2 h. After cooling, it is diluted to a final volume of 100 ml. Stored in a brown bottle, the solution is stable for several months.

Borate buffer: 50 ml of 0.4 Mol/l boric acid are mixed with 43.9 ml of 0.4 Mol/l sodium hydroxide (N. B.: The buffer has to be free of potassium chloride, since potassium causes a cloudy precipitation of phosphotungstic acid).

Stock uric acid standard (0.5 mMol/l): 8.4 mg uric acid are dissolved in 100 ml of a 0.1 mMol/l Na_2HPO_4 solution.

Procedure. The borate phosphotungstic mixture is rather instable with a half life period of only 20 min. Therefore, for every sample we mix a fresh solution with a pipette apparatus containing two glass syringes with a volume ratio of 16:1 (16 parts of buffer are added to one part of phosphotungstic acid). A pH of about 8.9 results which is the most optimal reaction condition. $1.0-1.5\times10^{-3}$ ml buffered phosphotungstic acid are mixed with $30-40\times10^{-6}$ ml sample or standard volume.

The optical density is measured after 20—50 min in the ultramicro cuvette against a reagent blank at 755 mμ using a Zeiss PMQ II spectrophotometer. Our ultramicro cuvette contains a capillary of black glass because silver as well as platinum diminishes the intensity of the colour.

Results

The parameters of uric acid (UA) handling in 51 micropuncture experiments are displayed in Table 1. With increasing distance of the puncture site from the glomerulus, TF/P_{UA} which is the ratio of UA concentration in tubular fluid and in plasma, increases. When water reabsorption and, therefore, decreasing intratubular solvent volume is taken into account by plotting TF/P_{UA} over TF/P_{Inulin}, it becomes apparent that per unit length the increase of TF/P_{UA} is larger than the increase of TF/P_{Inulin} in more than 80 % of the samples (Fig. 1). This indicates net UA secretion in these experiments. Furthermore, up to 35 % of UA in rat plasma is bound to plasma proteine [7]. Therefore, UA concentration in plasma water is lower than measured in total plasma and the increase in TF/P_{UA} compared to TF/P_{Inulin} is even higher than documented in Fig. 1.

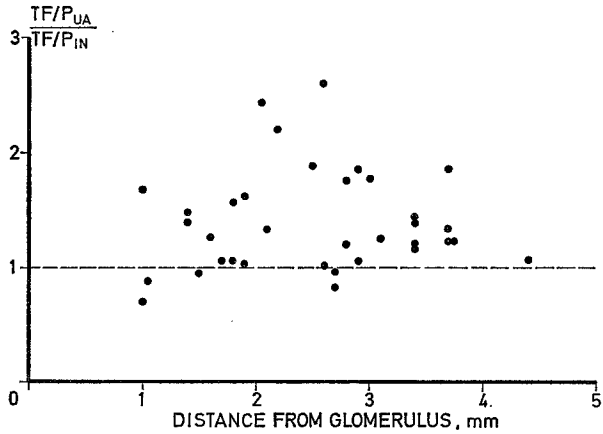

Fig. 1. Uric acid *(UA)* secretion in rat proximal tubules under free flow conditions. The increase of *UA* concentration in the tubular fluid *(TF)* in relation to plasma concentration *(P)* is corrected for concentration changes due to water movement by the ratio TF/P_{Inulin}

Table 1. *Uric acid concentrations in the tubular fluid of the rat proximal tubule under free flow conditions*

Rat No.	Distance from glomerulus mm	Uric acid			TF/P_{Inulin}	$\frac{TF/P_{UA}}{TF/P_{In}}$
		TF mMol/l	P mMol/l	TF/P		
5	—	0.25	0.085	3.0	1.75	1.72
	—	0.19	0.085	2.3	1.6	1.44
	—	0.28	0.085	3.4	2.4	1.42
	—	0.25	0.085	2.8	1.3	2.15
	—	0.33	0.085	3.9	3.1	1.26
	—	0.33	0.085	3.9	3.15	1.24
10	2.9	0.30	0.13	2.3	2.2	1.04
	1.0	0.20	0.13	1.55	0.92	1.68
	1.4	0.21	0.13	1.64	1.21	1.4
	4.2	0.35	0.13	2.75	2.6	1.06
	1.05	0.17	0.13	1.28	1.46	0.88
11	—	0.37	0.125	3.0	3.0	1.0
	—	0.17	0.125	1.35	1.75	0.77
	1.9	0.35	0.125	2.8	2.75	1.02
	—	0.37	0.125	3.0	1.55	1.04
	1.8	0.26	0.125	2.1	2.0	1.05
14	2.1	0.30	0.125	2.36	1.77	1.33
	3.2	0.28	0.125	2.24	1.6	1.4
	2.7	0.24	0.125	1.9	1.07	0.96
	1.6	0.32	0.125	2.52	2.0	1.26
	—	0.22	0.125	1.78	1.38	1.29
	3.7	0.46	0.125	3.7	2.0	1.85
	2.7	0.21	0.125	1.67	2.05	0.81
	3.1	0.30	0.125	2.42	1.05	1.24
	—	0.25	0.125	2.0	2.2	0.91
	2.6	0.27	0.125	2.15	2.15	1.0
15	2.6	0.48	0.10	4.8	1.85	2.6
	2.05	0.43	0.10	4.3	1.75	2.43
	2.2	0.27	0.10	2.7	1.35	2.0
	2.9	0.20	0.10	2.0	1.08	1.85
	2.8	0.30	0.10	3.0	2.5	1.2
	1.0	0.11	0.10	1.1	1.6	0.69
	2.5	0.37	0.12	3.2	1.7	1.88
	2.8	0.35	0.125	2.8	1.6	1.75
19	3.4	0.13	0.04	3.2	2.7	1.10
	3.4	0.14	0.04	3.5	2.45	1.43
	3.7	0.13	0.04	3.2	2.4	1.33
	3.0	0.17	0.04	4.25	2.4	1.77
	1.9	0.11	0.04	2.75	1.7	1.62
	—	0.15	0.04	3.7	2.1	1.76
	1.4	0.11	0.04	2.75	1.85	1.48
	—	0.17	0.04	4.35	3.0	1.45
	1.8	0.14	0.04	3.45	2.2	1.57

Table 1 (Continued)

Rat No.	Distance from glomerulus mm	Uric acid TF m/Moll	P mMol/l	TF/P	TF/P_{Inulin}	$\frac{TF/P_{UA}}{TF/P_{In}}$
21	3.4	0.28	0.09	3.14	2.7	1.16
	—	0.31	0.09	3.44	2.8	1.23
	3.75	0.34	0.09	3.85	3.15	1.22
	—	0.36	0.09	4.0	3.0	1.33
	3.7	0.35	0.09	3.9	3.2	1.22
	1.5	0.20	0.09	2.2	2.35	0.94
	—	0.24	0.09	2.7	2.78	0.97
	1.7	0.20	0.09	2.2	2.1	1.05

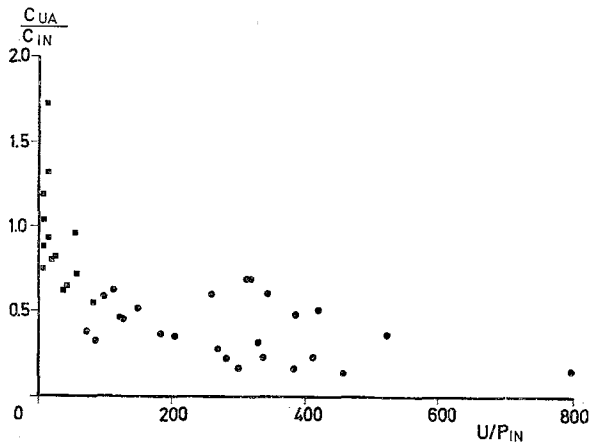

Fig. 2. Clearance ratio of uric acid *(UA)* and inulin in correlation to the diuretic state. Antidiuresis (•); osmotic diuresis (▪)

In Table 2 results of 31 clearance experiments are summarized. The data during antidiuresis (U/P_{Inulin} between 100 and 800) are spontaneous variations during experiments when only Ringer's solution was infused at 0.1 ml/kg × min. Measurements below U/P_{Inulin} of 100 were obtained during osmotic diuresis when a uric acid-mannitol solution (mannitol 136 mMol/l, uric acid 12 mMol/l, Na_2HPO_4 54 mMol/l, inulin 2 mg-%) was infused at 0.6 ml/kg × min. During antidiuresis the clearance of UA is well below that of inulin. The clearance ratio of UA to inulin ranges between 0.13 and 0.69. As demonstrated in Fig. 2 this ratio has a tendency

Table 2. *Uric acid and inulin clearance ratios in rats*

Rat No.	Uric acid		U/P_{Inulin}	$\frac{C_{UA}}{C_{In}}$
	Urine mMol/l	Plasma mMol/l		
2	5.354	0.093	125	0.46
	6.544	0.093	115	0.62
3	11.898	0.208	125	0.46
	5.949	0.208	75	0.38
5	4.938	0.083	100	0.59
	2.559	0.089	87.5	0.33
12	5.741	0.092	282	0.22
13	6.484	0.083	149	0.52
15	24.689	0.112	318	0.69
16	21.535	0.102	420	0.50
	18.441	0.100	387.5	0.48
	22.368	0.102	315	0.69
	19.988	0.137	344	0.60
	22.012	0.140	261	0.60
23	8.209	0.068	800	0.15
	12.671	0.068	525	0.36
24	4.366	0.058	337	0.23
	3.879	0.058	185	0.36
25	6.187	0.065	414	0.23
	4.616	0.065	205	0.35
26	4.7	0.095	300	0.16
27	7.2	0.065	330	0.32
28	6.2	0.089	270	0.27
	5.6	0.089	385	0.16
	5.2	0.089	460	0.13
30	0.81	0.018	82	0.55
	1.93	0.036	56	0.96
	1.05	0.196	4.5	1.19
	1.26	0.263	4.6	1.04
	1.82	0.340	6.1	0.88
	2.29	0.502	6.1	0.75
31	5.27	0.214	39	0.63
	4.30	0.202	26	0.82
	3.50	0.197	22	0.81
	3.16	0.202	17	0.92
	4.02	0.191	16	1.31
	3.93	0.167	14	1.68
32	2.19	0.137	25	0.64
	2.72	0.066	58	0.71

to increase when U/P_{Inulin} decreases in the course of a developing diuresis. In severe osmotic diuresis UA clearance approaches or even exceeds inulin clearance.

Discussion

Chemical Ultramicro Analysis. Our method allows quantitative measurement of as little as 10^{-12} Mols uric acid. The method, therefore, is sensitive enough to measure uric acid at a concentration of 0.1 mMol/l in a 10 nanoliter sample (10×10^{-9} l). The method is subject to a standard deviation of less than $\pm 5\%$ of the mean.

The redox reaction with phosphotungstic acid is not influenced significantly by any other substance present in the normal rat plasma or urine. Glycine or cystein increase the extinction coefficient at concentrations higher than 10 mMol/l. However, since normal plasma concentrations of these amino acids in rats are about 0.1 mMol/l, their influence on the reaction is negligible.

It should be emphasized that certain metals like silver, aluminum, iron or copper are able to reduce phosphotungstic acid. Therefore, during analysis the contact of samples and reagents with any metal has carefully to be avoided. This also includes the microcuvette for photometric readings. Even in a platinum cuvette colour already developed is reduced. We got the most satisfying results by using a tube of black capillary glass as a microcuvette [17].

Uric Acid Transport. As in humans and dogs [1,8,15,19], the clearance between UA and inulin in rats is also lower than 1.0 under normal conditions. In contrast to former concepts the site of this net reabsorption is not the proximal tubule. On the contrary, along the proximal convolution UA undergoes net secretion: The tubular load of UA at the end of the proximal convolution is significantly higher than the filtered load. Therefore, net reabsorption of UA must take place in a more distal part of the nephron. The localization of this process is described in more detail in a following paper [13].

However, it is apparent from this study and previous observations in the literature [15,20] that the reabsorptive process is dependent on the state of diuresis. With increasing diuresis the clearance of UA tends to approach inulin clearance and in an excessive osmotic diuresis may even exceed inulin clearance [9,18]. These findings suggest that the net reabsorption of UA takes place somewhere in the distal part of the nephron where intratubular flow velocity and therefore contact time vary in proportion to the diuretic state. During massive osmotic diuresis, tubular flow velocity is increased all along the nephron. Reduced volume

reabsorption and therefore lower concentration gradients of UA minimize the reabsorption of this substance and the increase of proximal load by UA secretion becomes detectable in the final urine.

References

1. Berger, L., Yü, T. F., Alsmon, A., Kupfer, S., Gutman, A. B.: Effect of reducing renal arterial blood pressure by balloon catheter on urate excretion in the dog. Proc. Soc. exp. Biol. (N. Y.) **115**, 5 (1964).
2. Caraway, W. T.: Determination of uric acid in serum by a carbonate method. Amer. J. clin. Path. **25**, 840 (1955).
3. Davis, B. B., Field, J. B., Rodnan, G. P., Kedes, L. H.: Lokalization and pyracinamide inhibition of distal transtubular movement of uric acid 2-^{14}C with a modified stop-flow technique. J. clin. Invest. **44**, 716 (1965).
4. Deetjen, P.: Tubulärer Transport schwacher organischer Säuren und Basen. In: Normale und pathologische Funktionen des Nierentubulus, S. 19—36. K. J. Ullrich u. K. Hierholzer (Ed.). Bern: Huber 1965.
5. Folin, O., Denis, W.: A new (colorimetric) method for the determination of uric acid in blood. J. biol. Chem. **13**, 469 (1912).
6. Führ, J., Kaczmarczyk, J., Krüttgen, C. D.: Eine einfache colorimetrische Methode zur Inulinbestimmung für Nieren-Clearance-Untersuchungen bei Stoffwechselgesunden und Diabetikern. Klin. Wschr. **33**, 729 (1955).
7. Greger, R., Lang, F., Deetjen, P.: Harnsäuretransport im Nierentubulus. In: Niere und Stoffwechsel, VII. Symp. Ges. Nephrol. Tübingen 1970, Hrsg. A. Bohle. Stuttgart: Schattauer (in press).
8. Gutman, A. B., Yü, T. F.: Renal function in gout, with a commentary on the renal regulation of urate excretion and the role of the kidney in the pathogenesis of gout. Amer. J. Med. **23**, 600 (1957).
9. — — Berger, L.: Tubular secretion of urate in man. J. clin. Invest. **38**, 1778 (1959).
10. — — Sirota, J. H.: A study by simultaneous clearance techniques of salicylate excretion in man. Effect of alkalinization of the urine by bicarbonate administration, effect of probenecid. J. clin. Invest. **34**, 711 (1955).
11. Hilger, H., Klümper, J. D., Ullrich, K. J.: Wasserrückresorption und Ionentransport durch die Sammelrohrzellen der Säugetierniere (mikroanalytische Untersuchungen). Pflügers Arch. ges. Physiol. **267**, 218 (1958).
12. Kessler, R. H., Hierholzer, K., Gurd, R. S.: Lokalization of urate transport in the nephron of mongrel and Dalmatian dog kidney. Amer. J. Physiol. **197**, 601 (1959).
13. Lang, F., Greger, R., Deetjen, P.: Handling of uric acid by the rat kidney. II. Microperfusion studies on bidirectional transport of uric acid in the proximal tubule. Pflügers Arch. (in preparation).
14. Lorentz, K., Berndt, W.: Enzymic determination of uric acid by a colorimetric method. Analyt. Biochem. **18**, 58 (1967).
15. Mudge, G. H., Cucchi, J., Platts, M., O'Conell, J. M. B., Berndt, W. O.: Renal excretion of uric acid in the dog. Amer. J. Physiol. **215**, 404 (1968).
16. Oelert, H., Bauman, K., Gekle, D.: Permeabilitätsuntersuchungen einiger schwacher organischer Säuren aus dem distalen Konvolut der Rattenniere. Pflügers Arch. **307**, 178 (1969).
17. Schneider, W., Greger, R.: An improved microcuvette for photometric measurements. Pflügers Arch. **311**, 268 (1969).

18. Skeith, M. P., Healy, L. A., Cutler, R. E.: Urate excretion during mannitol and glucose diuresis. J. Lab. clin. Med. **70**, 213 (1967).
19. Wirz, H.: Tubulusfunktion und Membrantransport der Harnkonzentrierung. Handbuch der Inneren Medizin, Bd. VIII/1, S. 115. Berlin-Heidelberg-New York: Springer 1968.
20. Yü, T. F., Berger, L., Kupfer, S., Gutman, A.: Tubular secretion of urate in the dog. Amer. J. Physiol. **199**, 1199 (1960).
21. Zins, G. R., Weiner, I. M.: Carriermediated entry of urate into proximal tubule of mongrel dog. Fed. Proc. **26**, 265 (1967).
22. — — Bidirectional urate transport limited to the proximal tubule in dog. Amer. J. Physiol. **215**, 411 (1968).

Prof. Dr. P. Deetjen
Physiologisches Institut der Universität
A-6020 Innsbruck, Schoepfstr. 41