

A New Method for Studying the Function of the Descending Part of the Proximal Tubule *in Vivo*

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The relative inaccessibility of the loop of Henle of superficial nephrons to micropuncture methods *in vivo* has previously prevented quantitative evaluation of its transport characteristics. The present method was designed to obtain information about the descending part of the proximal tubule (pars recta) *in vivo* by a modified stopped-flow microperfusion technique. From the results it can be assumed that the maximal sodium concentration difference between tubular and peritubular fluid is constant along the entire proximal tubule. In addition, the method can be applied to other tubular segments also inaccessible to micropuncture studies.

METHODS

Male rats, 180-220 g body wt, were prepared for micropuncture experiments by methods previously used in our laboratory(1,2). In antidiuretic animals U/P_{osmol} was in the range of 3.4-4.5, whereas in osmotic diuresis (induced by infusion of 5 ml of 20% mannitol per hour over a period of 2-3 hr) urine osmolality was very close to plasma osmolality.

Since the descending part of the proximal tubule does not approach the surface of the kidney, the micropuncture method shown in Fig. 1 was used. With double-barreled pipetes the second or third loop of the proximal convolution was punctured. A test solution (Na 110 meq/liter, polyethylene glycol 7.6%, osmolality 300 mOsm/liter) of about 1×10^{-6} ml was injected between columns of heavy castor oil (part a in Fig. 1). By filling the total proximal convolution with castor oil the test solution was then moved into the descending part of the proximal tubule, which extends from the cortex to the outer stripe of the outer

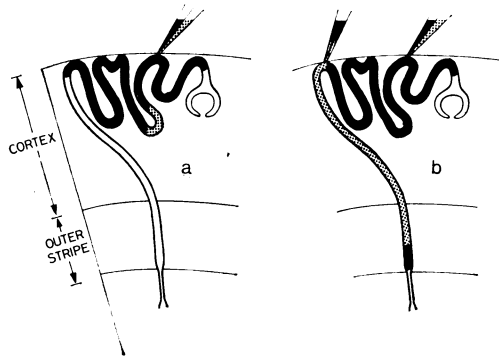


FIG. 1. Schematic illustration of the method by which the transport capacity of the descending part of the proximal tubule can be measured *in vivo* (see Methods for explanation).

medulla (part b in Fig. 1). After a contact time of 2–3 min the injected fluid was reaspirated from the last loop of the proximal convolution. Experiments were accepted only when reaspiration could be performed in less than 2 sec and the oil droplet from the descending part reappeared at the collecting pipette.

In mannitol diuresis tubular fluid from the proximal convolution was used as test solution. Plasma and tubular fluid samples were analysed for Na, K, and osmolality(21).

RESULTS AND DISCUSSION

In four rats the length of the proximal tubule was determined by microdissection. The data (convoluted part: 4.69 ± 0.08 mm, $n = 25$; descending part: 3.03 ± 0.08 mm, $n = 40$) are in substantial agreement with measurements in 200 to 280-g rats(3–6). Like Maunsbach(7) we prefer the term “descending part” instead of “straight part” or “pars recta”(8) because the descending part is not straight.

In 11 antidiuretic rats (Fig. 2) sodium concentration of tubular fluid reaspirated from the descending part of the proximal tubule was 126.1 ± 1.2 meq/liter. The sodium concentration of arterial blood (carotid artery) was 140.0 ± 0.3 meq/liter.

The potassium concentration of the descending part ranged from 3.3 to 4.4 meq/liter with a mean value of 3.80 ± 0.06 meq/liter (mean plasma potassium 4.11 ± 0.09 meq/liter).

To obtain information about the maximal concentration difference against which sodium can be pumped under steady-state conditions from tubular fluid of the descending part to peritubular fluid, sodium concentration of peritubular fluid must be calculated. In superficial nephrons of the rat the descending part of the proximal tubule extends from the cortex to the boundary between the outer and inner stripe of the outer zone(9). According to cryoscopic measurements of osmolality of renal tissue(10–12) the descending part of the proximal

tubule is surrounded by a peritubular fluid with a mean osmolality of about 340–350 mOsm/liter.

To test this assumption, osmolality of tubular fluid was determined in 5 of the 11 antidiuretic rates (Fig. 3). In the proximal convolution under steady-state conditions the mean osmolality (302.3 ± 3.4 mOsm/liter) was not significantly different from plasma osmolality (carotid artery). However, the mean osmolality of tubular fluid from the descending part (340.0 ± 3.7 mOsm/liter) was significantly higher ($P < 0.001$) than osmolality in the proximal convolution and was very close to the expected data calculated from tissue measurements.

Assuming that the sodium chloride in peritubular fluid of cortex and outer zone is about 90% responsible for osmolality, one can calculate a peritubular sodium concentration of about 150–160 meq/liter surrounding the descending part of the proximal tubule. From measured and calculated data it can be postulated that, under steady-state conditions, the maximal concentration difference for sodium between tubular fluid of the descending part and surrounding peritubular fluid is in the range of 30 meq/liter. This concentration difference for sodium is similar to the maximal concentration difference in the proximal convolution, where limiting intratubular sodium concentration is 113 meq/liter(2,13).

Since the calculation of peritubular sodium concentration is open to some criticism, the method was tested in osmotic diuresis. Under those conditions the mean sodium concentration of reaspirated fluid from the descending part was 113.9 ± 2.1 meq/liter (Fig. 2). Due to a washout of the corticomedullary gradient(11), one can assume that the sodium concentration in peritubular fluid surrounding the descending part is similar to plasma sodium concentration (139.3 ± 0.9 meq/liter). Again, the calculated concentration difference for sodium of about 25 meq/liter is equal to the concentration difference in the proximal convolution under similar conditions(14–16). Both in antidiuresis and in osmotic diuresis the tubular epithelium of the descending part of the proximal tubule is able to transport sodium against a concentration difference of 25–30 meq/liter. The same concentration difference can be established by the tubular epithelium of the proximal convolution independent of the peritubular sodium concentration(17). The results represented here suggest that the transport capacity for sodium under steady-state conditions is constant along the entire proximal tubule.

Marked differences between the convoluted and descending part of the proximal tubule were demonstrated by light- and electron-microscopic studies(7, 18–22). The main difference is the lack of “basal labyrinth”(23) in the descending part which is typical for epithelia with little volume reabsorption(21,22). On the other hand, calculated per membrane surface the content of high-energy substrates is much higher in the descending part than in the convoluted part(20).

The morphological findings correlate well with physiological data: 1. Free flow micropuncture experiments have shown that 50–60% of the glomerular filtrate is reabsorbed by the proximal convolution and only 15–20% by the loop of Henle including the descending part of the proximal tubule. Thus, a lower rate of fluid

reabsorption in the descending part compared with the convoluted part had been suspected(24-26). In *in vitro* experiments with isolated tubules, reabsorption rate of the descending part was found to be approximately one-half as great as reabsorption of the convoluted tubule(27). 2. Under steady-state conditions with practically infinite contact time (2-3 min) the tubular epithelium of the descending part is able to build up a concentration difference for sodium *in vivo* which is similar to the concentration difference in the convoluted part.

Although the primary purpose of these studies was to learn about the transport capacity of the descending part of the proximal tubule, the method described can be applied *in vivo* to other tubular segments which do not approach the surface of the kidney and are, therefore, inaccessible for direct micropuncture studies.

In summary, in rat kidney a test solution (Na 110 meq/liter; polyethylene glycol 7.6%; osmolality 300 mOsm/liter) was injected from the proximal convolution into the descending part of the proximal tubule (pars recta). Castor oil on both sides of the test solution prevented contamination with tubular fluid from other segments. After a contact time of 2-3 min the injected fluid was reaspirated from the last loop of the proximal convolution. In antidiuresis

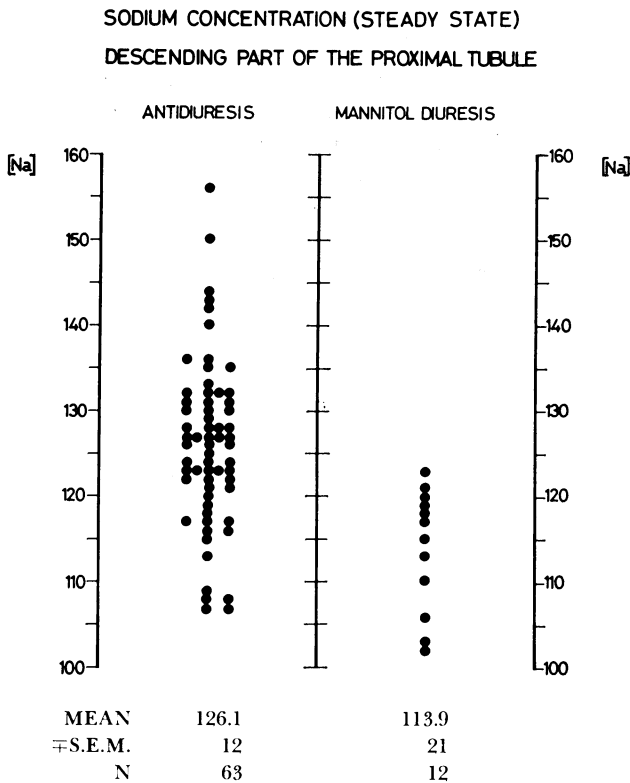


FIG. 2. Measurements of sodium concentration of tubular fluid reaspirated from the descending part of the proximal tubule.

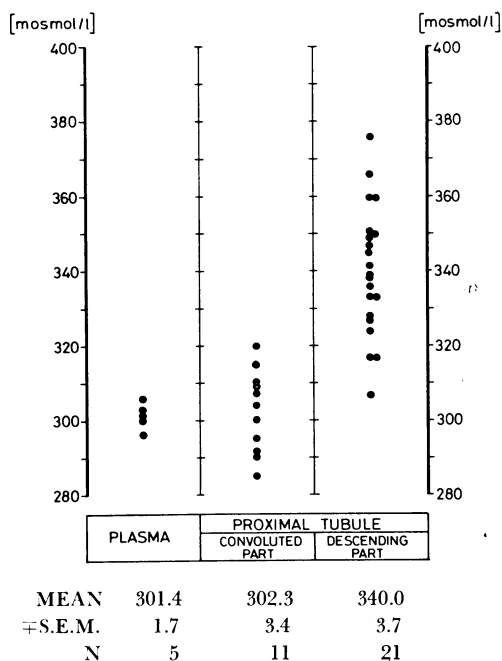


FIG. 3. Osmolality of plasma and tubular fluid (steady-state conditions).

results of microanalysis of reaspirated fluid were as follows: Na 126.1 ± 1.2 meq/liter (113.9 ± 2.1 meq/liter in osmotic diuresis); potassium 3.80 ± 0.06 meq/liter; osmolality 340.0 ± 3.7 mOsm/liter. It was concluded: 1. The maximal concentration difference (steady state) for sodium between tubular and peritubular fluid (25–30 meq/liter) in antidiuresis as well as in osmotic diuresis was constant along the entire proximal tubule. 2. The tubular fluid in the descending part of the proximal tubule of superficial nephrons was in osmotic equilibrium with the surrounding tissue. 3. The measured increase of osmolality of reaspirated fluid was due to influx of solute (Na) or efflux of water or both. 4. Assuming a small (lumen-negative) or insignificant transtubular potential difference, sodium was actively transported by the tubular epithelium of the descending part of the proximal tubule. 5. The method can be applied to other tubular segments which are inaccessible for micropuncture studies *in vivo*.

REFERENCES

1. Hierholzer, K., Wiederholt, M., und Stolte, H. Hemmung der Natriumresorption im proximalen und distalen Knovolut adrenaletomierter Ratten. *Pflügers Arch. Physiol.* **291**, 43 (1966).
2. Wiederholt, M., und Wiederholt, B. Der Einfluss von Dexamethason auf die Wasser und Elektrolytausscheidung adrenaletomierter Ratten. *Pflügers Arch. Physiol.* **302**, 57 (1968).

3. Capek, K., Fuchs, G., Rumrich, G., and Ullrich, K. J. Harnstoffpermeabilität der corticalen Tubulusabschnitte von Ratten in Antidiurese und Wasserdiurese. *Pflügers Arch. Physiol.* **290**, 237 (1966).
4. Deetjen, P. Mikropunktionsuntersuchungen zur Wirkung von Furosemid. *Pflügers Arch. Physiol.* **284**, 184 (1965).
5. Gottschalk, C. W., and Mylle, M. Micropuncture study of pressures in proximal tubules and peritubular capillaries of the rat kidney and their relation to ureteral and renal venous pressures. *Amer. J. Physiol.* **185**, 430 (1956).
6. Rouffignac, De, C., and Morel, F. Etude par microdissection de la distribution et de la longueur des tubules proximaux dans le rein de cinq espèces de rongeur. *Arch. d'Anatom. Microscop.* **56**, 123 (1967).
7. Maunsbach, A. B. Observation on the segmentation of the proximal tubule in the rat kidney. Comparison of results from phase contrast, fluorescence and electron microscopy. *J. Ultrastructure Res.* **16**, 239 (1966).
8. Möllendorf, W. Der Exkretionsapparat, in "Handbuch der mikroskopischen Anatomie des Menschen," Bd.VII/1. Springer, Berlin, 1930.
9. Kriz, W. Der architektonische und funktionelle Aufbau der Rattenniere. *Z. Zellforsch.* **82**, 495 (1967).
10. Bray, G. A. Freezing point depression of rat kidney slices during water diuresis and anti-diuresis. *Amer. J. Physiol.* **199**, 915 (1960).
11. Heller, J., and Vostal, J. The plasma sodium concentration: a factor influencing the osmolality of the fluid of the renal medullary tissue. *Physiol. Bohemoslov.* **16**, 418 (1967).
12. Wirz, H., Hargitay, B., and Kuhn, W. Lokalisation des Konzentrierungsprozesses in der Niere durch direkte Kryoskopie. *Helv. Physiol. Arch.* **9**, 196 (1951).
13. Hierholzer, K., Wiederholt, M., Holzgreve, H., Giebisch, G. Klose, R. M., and Windhager, E. E. Micropuncture study of renal concentration gradients of sodium and potassium in adrenalectomized rats. *Pflügers Arch. Physiol.* **285**, 193 (1965).
14. Stolte, H., Wiederholt, M., Fuchs, G., and Hierholzer, K. Time course of development of transtubular sodium concentration differences in proximal surface tubules of the rat kidney. *Pflügers Arch. Physiol.* **313**, 252 (1969).
15. Ullrich, K. J., Schmidt-Nielsen, B., O'Dell, R., Pehling, C., Gottschalk, C. W., Lassiter, W. E., and Mylle, M. Micropuncture study of composition of proximal and distal tubular fluid in rat kidney. *Amer. J. Physiol.* **204**, 527 (1963).
16. Windhager, E. E., and Giebisch, G. Micropuncture study of renal tubular transfer of sodium chloride in the rat. *Amer. J. Physiol.* **200**, 581 (1961).
17. Baldamus, C. A., Hierholzer, K., Rumrich, G., Stolte, H., Uhlich, E., Ullrich, K. J., and Wiederholt. Natriumtransport in den proximalen Tubuli und den Sammelrohren bei Variation der Natriumkonzentration im umgebenden Interstitium. *Pflügers Arch. Physiol.* **310**, 354 (1969).
18. Caulfield, J. B., and Trump, B. F. Correlation of ultrastructure with function in the rat kidney. *Amer. J. Pathol.* **40**, 199 (1962).
19. Ericsson, J. L. E. Electron microscopy of the normal tubule. Proc. 3rd. Int. Congr. Nephrol., Washington, 1966, 2, 1 (1967).
20. Sitte, H. Beziehungen zwischen Zellstruktur und Stofftransport in der Niere. 2.Symp.Dtsch. Ges. Naturforsch. Ärzte "Sekretion und Exkretion," Reinhardtsbrunn, 1964, p. 343. Springer, Berlin-Heidelberg-New York, 1965.
21. Thoenes, W. Die Mikromorphologie des Nephrons in ihrer Beziehung zur Funktion. II: Funktionseinheit: Henlesche Schleife-Sammelrohr. *Klin. Wschr.* **39**, 827 (1961).
22. Thoenes, W. Transportwege in den Harnkanälchen der Säugerniere. 2.Symp.Dtsch.Ges. Naturforsch. Ärzte "Sekretion und Exkretion," Reinhardtsbrunn, 1964, p. 315. Springer, Berlin-Heidelberg-New York, 1965.

23. Ruska, H., Moore, D. H., and Weinstock, J. The base of the proximal convoluted tubule cells of rat kidney. *J. Biophys. Biochem. Cytol.* **3**, 249 (1957).
24. Giebisch, G., and Windhager, E. E. Renal tubular transfer of sodium, chloride and potassium. *Amer. J. Med.* **36**, 643 (1964).
25. Gottschalk, C. W. Renal tubular function: lessons from micropuncture. *Harvey Lectures* **58**, 99 (1963).
26. Walker, A. M., Bott, P. A., Oliver, L., and MacDowell, M. C. The collection and analysis of fluid from single nephrons of the mammalian kidney. *Amer. J. Physiol.* **134**, 580 (1941).
27. Burg, M. B., and Orloff, J. Control of fluid absorption in the renal proximal tubule. *J. Clin. Invest.* **47**, 2016 (1968).