Pflügers Arch. 368, 45–48 (1977)

Pflügers Archiv
European Journal of Physiology

Stationary Microperfusion Study of Phosphate Reabsorption in Proximal and Distal Nephron Segments

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Summary. Micropuncture studies demonstrate phosphate reabsorption in proximal tubules and between the late proximal and early distal convoluted tubule accessible to micropuncture. To further define the sites of phosphate reabsorption, the stationary microperfusion technique was applied to proximal and distal nephron segments. Phosphate reabsorption was evaluated in superficial loops of proximal tubules, descending segments beyond late proximal tubules accessible to micropuncture, ascending segments up to the point of micropuncture in the distal tubule, and superficial loops of distal tubules of thyroparathyroidectomized rats. Microperfusates of 1.3 or 2.6 nl (100 mmol/l mannitol, 100 mmol/l NaCl, ^{32}P-phosphate and ^{3}H-inulin) were injected and then withdrawn after contact times of 2–108 s. Phosphate recovery relative to that of inulin was determined. A steep exponential decline of phosphate recovery (R) with increasing contact time (t) was observed in the superficial proximal tubule and descending segments. The slopes of the logarithmic regressions ($^{10}\log R)/t$, ± SEM) were: -1.68 ± 0.33 and $-1.21 \pm 0.24\ \text{min}^{-1}$ in superficial proximal tubules and descending segments respectively. In contrast, no significant decline in phosphate recoveries (-0.02 ± 0.04 and $+0.11 \pm 0.10\ \text{min}^{-1}$) was apparent in the ascending segments and distal tubule. It is concluded that phosphate is reabsorbed in the proximal convoluted tubule and adjacent descending segments of the superficial nephron and that there is no significant phosphate reabsorption in distal convoluted tubules and adjacent ascending segments.

Key words: Phosphate – Renal transport – Proximal tubule – Distal tubule – Loop of Henle.

INTRODUCTION

The nephron sites of phosphate reabsorption have been investigated with several techniques. Agreement exists that the proximal convoluted tubule is a principal site of phosphate reabsorption. In addition, significant amounts of phosphate are reabsorbed beyond the late proximal tubule accessible to micropuncture [6]. This reabsorption is particularly evident in thyroparathyroidectomized animals [5–7]. However, controversy exists in regard to the site of this phosphate reabsorption. In free flow micropuncture studies, the difference in phosphate recovery between superficial distal convoluted tubules and urine has been interpreted as evidence for phosphate reabsorption in the distal nephron and collecting system [3]. Microinjection studies, on the other hand, do not demonstrate significant phosphate reabsorption in the distal nephron and collecting system [2, 5, 9]. Both free flow micropuncture and microinjection studies indicate that phosphate reabsorption occurs between the late proximal and early distal tubule accessible to micropuncture [2, 5, 8]. The present studies were undertaken to further localize the sites of phosphate reabsorption beyond the proximal tubule accessible to micropuncture.

The technique of stationary microperfusion was chosen because it allows detection of small fluxes by prolonging the duration of fluid in contact with the epithelium. Furthermore, microperfusate may be moved into nephron segments which are not otherwise accessible to micropuncture. To facilitate detection of phosphate reabsorption, rats were thyroparathyroidectomized.

METHODS

Munich Wistar rats (150–200 g) were anesthetized with inactin® (Byk Gulden, 120 mg/kg B.W.). The temperature of the animals was maintained at 37° C. After tracheostomy, the blood supply to

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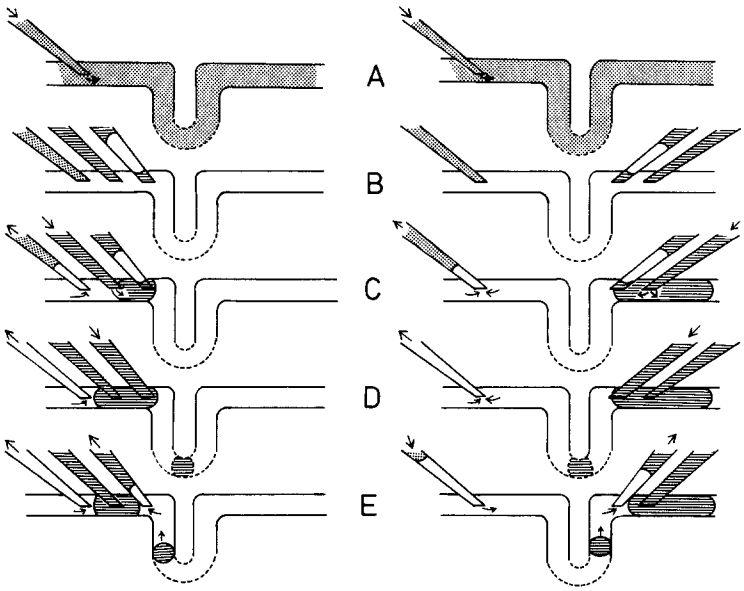

Fig. 1 A–E
Experimental procedure of stationary microperfusion in descending (left panel) and ascending (right panel) segments of "Loops of Henle".
(A) Identification of nephron with Lissaminegreen;
(B) puncture;
(C) introduction of oil block;
(D) injection of test droplet;
(E) recovery of test droplet

the thyroid and parathyroid glands was interrupted by ligation of the arteries and veins. Then the parathyroid glands were identified under the microscope and destroyed by thermocautery and finally the remainder of the thyroid gland was cauterized. Animals were infused with 3.3 ml/h isotonic saline through a catheter in the right jugular vein. After 2 h, the left kidney was exposed by a flank incision and prepared for micropuncture.

The composition of the microperfusate was: 100 mmol/l NaCl, 100 mmol/l mannitol, 1 mmol/l ^{32}P-phosphate, ^{3}H-inulin and 1 g/l Lissamine green. No buffer was added so that the injected fluid would rapidly assume the pH of the tubule segment. The stationary microperfusion was performed with 3 micropuncture pipettes. The first pipette was filled with isotonic saline containing 1 g/l Lissamine green dye. The second pipette contained Sudan black stained castor oil. The third pipette was filled with castor oil and then 1.3 or 2.6 nl microperfusate was introduced from calibrated pipettes. The tip was sealed with castor oil. Phosphate reabsorption was evaluated in proximal (series 1) and distal (series 2) nephron segments. In series 1 a, phosphate reabsorption in the proximal convoluted tubules of the superficial nephron was determined as follows: The first pipette was inserted into a proximal convoluted tubule and the subsequent nephron loops were identified by injection of small quantities of the dye. The second pipette was inserted close to the first and a large column of oil was injected. The oil was held in place by aspiration of tubule fluid proximal to the oil column with the first pipette. The oil column was split by injection of microperfusate with the third pipette. 1.3 nl of microperfusate was injected in all experiments except for 2 experiments in series 2b in which 2.6 nl was injected. After elapse of 2–108 s, the microperfusate was aspirated with the third pipette. In series 1b, phosphate reabsorption beyond the point of micropuncture in the late proximal tubule was evaluated. The procedure was identical to that in series 1a (accessible loops of proximal tubules) with the exception that the third pipette was inserted into the last loop accessible to micropuncture and that the microperfusate was pushed into the descending segments of the nephron by the second pipette. The drop of microperfusate was held in place so that the end of the drop was visible on the surface (Fig. 1).

In series 2b, phosphate reabsorption in the ascending segments up to the point of micropuncture in the distal tubule was evaluated. The second pipette was inserted distal to the third pipette in the distal convoluted tubule. Oil was injected by the second pipette and suction was applied to the first pipette. When the oil block in the distal tubule moved upstream and reached the third pipette, the microperfusate was injected followed by additional oil. Thus, microperfusate was pushed retrograde into the ascending segments of the nephron. When the microperfusate was aspirated by the third pipette, fluid was injected into the proximal tubule by the first pipette to facilitate recovery (Fig. 1). In series 2a, phosphate reabsorption in the distal convoluted tubule was evaluated. Stationary microperfusion was performed in the distal convoluted tubule in the same manner as described above except that the droplet was not pushed upstream.

Radioactivity from ^{32}P-phosphate (NEN, Boston, S.A. 200 mCi/mmol) and ^{3}H-inulin (NEN, S.A. 220 mCi/g) was counted in a Searle liquid scintillation counter with windows adjusted to reduce spillover to less than 5%. The scintillation fluid contained 6 g Permablend II, 667 toluene, and 333 ml Triton X® (Packard). The activity injected was about 15× background for ^{3}H and 30× background for ^{32}P. Samples with ^{3}H activity less than 3× background were discarded. Counting times were adjusted to reduce counting errors to less than 3%. Phosphate recoveries (R) were calculated from concentrations of phosphate (P) and inulin (In) in the injected fluid (P_0, In_0) and collected fluid (P, In): $R = (P/In)/(P_0/In_0)$. Linear regressions of least square fit were calculated from the contact times (t) and the decadic logarithms of the recoveries: $^{10}\log R = a \cdot t + b$.

The mean recovery of inulin was similar in all 4 series and was 0.41 ± 0.25 (range 0.12–1.0). To test whether incomplete recovery affected the results, $(P/P_0/In/In_0)$ was plotted versus the inulin recovery (In/In_0). In series 1 a, and 1b, the values were corrected for the time course. The slope for all microperfusions was close to zero (0.007) and no correlation was apparent ($r = 0.01$).

RESULTS

The results of stationary microperfusion from proximal and distal nephron segments are summarized in Figure 2. In series 1a, (proximal convoluted tubule) phosphate recovery as a function of time decreased markedly. In 12 experiments, the slope of the regression was −1.68 ± 0.33 (log scale, ± SEM) with a correlation coefficient of 0.85, $P < 0.001$. The inter-

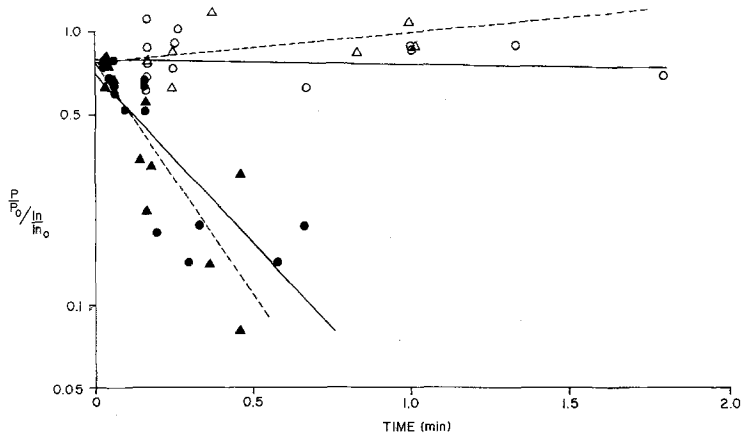

Fig. 2
Phosphate recovery as a function of time.
Ordinate: Fractional phosphate recovery.
Abscissa: Exposure time. Closed triangles: proximal convoluted tubules, closed circles: descending segments, open circles: ascending segments, open triangles: distal convoluted tubules. Broken lines: linear regressions of data points from proximal and distal convoluted tubules respectively, solid lines: linear regressions of data points from descending and ascending segments of "Loops of Henle" respectively

cept, -0.11 ± 0.08 (log scale, $\pm$ SEM), or 78% recovery, was not significantly different from full recovery. The time required for reabsorption of one-half of the injected phosphate was 11 s as calculated from the slope of the regression. In series 1b (descending segments beyond the late proximal tubule accessible to micropuncture), phosphate recovery as a function of time decreased similar to that observed in series 1a. In 15 experiments, the slope of the regression was -1.21 ± 0.24 with a correlation coefficient of 0.81, $P < 0.001$. The intercept, -0.15 ± 0.07, or 71% recovery, was significantly less than full recovery, $P < 0.05$. The half time for reabsorption was 15 s again calculated from the slope of the regression.

In series 2a (distal convoluted tubule), phosphate recovery as a function of time did not significantly change. In 7 experiments, the slope of the regression was $+0.11 \pm 0.10$ with a regression coefficient of 0.43, NS. The intercept, -0.13 ± 0.07, or 74% recovery was not significantly different from full recovery. In series 2b (ascending segments up to the point of micropuncture in the distal tubule), phosphate recovery as a function of time was not significantly changed. In 13 experiments, the slope of the regression was -0.02 ± 0.04 with a correlation coefficient of 0.12 NS. The intercept, -0.10 ± 0.03, or 79% recovery, was significant, $P < 0.05$.

DISCUSSION

The present study was designed to evaluate the presence of phosphate reabsorption in proximal and distal segments of superficial nephron. Disappearance of luminal tracer, however, could be due to both reabsorption and mixing with the cellular pool including binding to brush border membranes. A previous stationary microperfusion study indicates that both phenomena contribute to the decline of luminal tracer concentration [1]. When both cold and labelled phosphate were added to luminal fluid, tracer phosphate concentration initially declined more rapidly than chemically determined phosphate concentration. After 10 s the concentration of labelled phosphate was approximately 50% of that for chemically determined phosphate. The further disappearance of labelled and chemically determined phosphate was similar. Thus, it is likely that labelled phosphate rapidly mixes with the cellular pool and that this is followed by relatively slow reabsorption. Accordingly, in the present studies, it is likely that the intercepts are the result of mixing with the cellular pool and the slopes are largely attributable to reabsorption.

The results in series 1a show that, as expected, phosphate reabsorption is avid in the proximal convoluted tubule. The results in series 1b were similar indicating the presence of phosphate reabsorption beyond the late proximal tubule accessible to micropuncture. The volume of the nephron segment between the late proximal and early distal nephron accessible to micropuncture is approximately 2–4 nl as estimated from flow rates and transit times. Thus, the injected volume, 1.3 nl, had contact with additional segments of the proximal convoluted tubule and most likely major portions of the pars recta. Conclusions in regard to the thin loop cannot be drawn from this study. The results demonstrate that phosphate reabsorption continues in the adjacent segments beyond the late proximal tubule accessible to micropuncture. This finding is in good agreement with the observation that both the pars convoluta and pars recta of the isolated proximal tubule are capable of reabsorbing phosphate [4].

In contrast, no evidence for phosphate reabsorption by the distal nephron was found even for contact times of 1 min. This observation supports similar results from microinjection studies where urinary

phosphate recovery was complete after injection into distal convoluted tubules [2, 5]. Microinjection results have been criticized since injection of fluid into the distal tubule increases tubule flow rates and thus the reduced contact time with the distal tubular epithelium might result in an underestimation of a low affinity transport process. The present finding demonstrates that the failure to show phosphate reabsorption in microinjection studies cannot be attributed to the decreased contact time. Similarly, there was no significant reabsorption of phosphate in the ascending segment up to the point of micropuncture in the distal tubule even for contact times exceeding 1 min. Again, this segment represents the early distal convoluted tubule and major portions of the thick ascending limb of the loop of Henle.

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Received September 14, 1976