

pH – DEPENDENCE OF PHOSPHATE ABSORPTION IN RAT RENAL PROXIMAL TUBULE

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Summary

Proximal tubular cell membrane potentials were measured in rat kidney in vivo and the response to luminal perfusion of 2mmolar phosphate (P_i) was studied. P_i transport was preferentially rheogenic at low pH (cotransport of $1H_2PO_4^-$ plus $2Na^+$) but preferentially electroneutral at high pH (cotransport of $1HPO_4^{--}$ plus $2Na^+$). The potential response as a function of pH conformed to a model which transports both $H_2PO_4^-$ and HPO_4^{--} indiscriminately and whose maximal transport capacity increases with increasing pH. Further kinetic experiments are required to definitely exclude separate transport systems for both ionised forms. Hypercapnic phosphaturia may be explained by a decreased maximal transport capacity of P_i at low luminal pH.

Introduction

Hypercapnia decreases inorganic phosphate (P_i) resorption in renal proximal tubules in vivo [1]. This observation was confirmed by micropuncture and microperfusion experiments [2, 3] which suggested that decreasing luminal pH reduced the Na^+ dependent P_i transport. Two explanations of this effect are possible: 1) If only HPO_4^{--} was transported, as was recently concluded from experiments on brush border membrane vesicles [4], P_i transport could decrease as the HPO_4^{--} concentration decreased with decreasing pH. 2) If the transport system transported both $H_2PO_4^-$ and HPO_4^{--} without discrimination [5] it could be that the transport velocity (v_{max}) decreased with decreasing luminal pH.

Some idea on whether only HPO_4^{--} or also $H_2PO_4^-$ is transported, can be obtained from electrophysiological experiments. If the cotransporter has two binding sites for Na^+ , as suggested by some experiments on brush border membrane vesicles [6] we expect that the cotransport of $1HPO_4^{--}$ plus $2Na^+$ is electroneutral and the cotransport of $1H_2PO_4^-$ plus $2Na^+$ is rheogenic, i.e. associated with a positive current flow from lumen to cell. Accordingly we

predict that in the former case the membrane potential across the brush border should not change during phosphate cotransport, while in the latter case it should decrease.

Study

We have therefore monitored the membrane potential of rat proximal tubular cells *in vivo* with microelectrodes, while perfusing the tubular lumen alternately with Ringers solution containing either zero or 2mmolar P_i . To investigate the transport of $H_2PO_4^-$ the pH of the perfusate (HCO_3^- free Ringer solution buffered with MES [7]) was lowered to 6.0 at which pH 83.6 per cent of the total P_i is present in form of $H_2PO_4^-$. Under those conditions a small but significant depolarisation was observed in response to P_i perfusion, mean value + 4.4mV, SD \pm 1.3mV ($n = 8$ tubules) [8]. That this depolarisation indeed reflected an excess of Na^+ ions which are cotransported together with $H_2PO_4^-$, was demonstrated by perfusing the tubular lumen with Na^+ -free choline Ringer solution. As shown in Figure 1 in the absence of Na^+ the potential response to P_i was abolished, the mean potential change in Na^+ -free solutions was + 0.4mV, SD \pm 1.2mV, $n = 6$ tubules. To test for the transport of HPO_4^{--} , the perfusate pH was increased to 8.5 by adding HCO_3^- but omitting all other buffers and equilibrating the solution with air instead of 5 per cent CO_2 . Under those conditions no significant cell depolarisation occurred in response to P_i , mean value 0.0mV, SD \pm 0.4mV $n = 11$ tubules. Since at pH 8.5 the $H_2PO_4^-$ concentration is only 2.3 per cent of total P_i these data suggest that HPO_4^{--} was predominantly transported in the latter case and that its transport was indeed electroneutral.

To obtain more insight into the relation between the transports of primary and secondary P_i we have studied the pH-dependence of the potential response to luminal P_i perfusion (2mmol/L) in greater detail.

The pH of the perfusate (HCO_3^- free, Na^+ Ringer solution) was varied in steps of 0.6 pH units using the non-permeable buffers MES, ACES and HEPES [7] in the acid, neutral and alkaline range respectively. To reduce scatter all tubules were first perfused with a pair of P_i -free and P_i -containing reference solutions of pH 6.0 and subsequently with the corresponding pair of test solutions of pH 6.6, 7.2 or 7.8, and all cell depolarisations were expressed in percentage of the potential changes observed at pH 6.0. Measurements in which the latter potential response was < 1.5 mV were discarded. The results are shown in Figure 2. It can be seen that the cell depolarisation decreased with increasing pH but did not follow the concentration of $H_2PO_4^-$ in the perfusate.

Discussion

This observation indicates that either:

1. $H_2PO_4^-$ transport follows the luminal $H_2PO_4^-$ concentration proportionately but the Na^+ to $H_2PO_4^-$ stoichiometry increases with increasing pH; or
2. $H_2PO_4^-$ transport does not follow the luminal $H_2PO_4^-$ concentration proportionately for one of the following reasons:

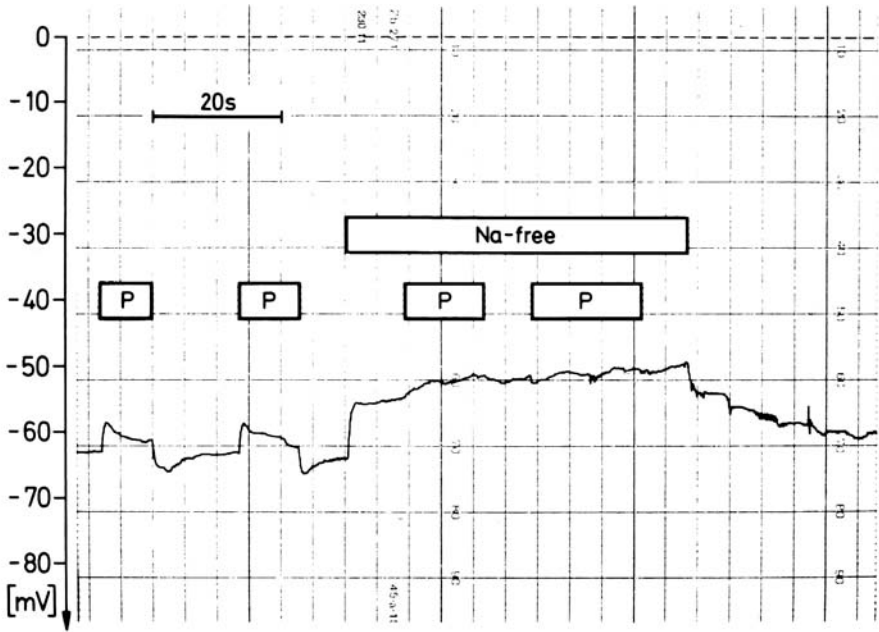


Figure 1. Trace record of proximal tubular cell membrane potential. Note that the response to luminal application of 2mmolar P_i (during marks: P) disappears in the absence of Na^+ (mark: Na^+ -free), indicating Na^+ -dependent rheogenic P_i absorption. The pH of all perfusates was 6.0

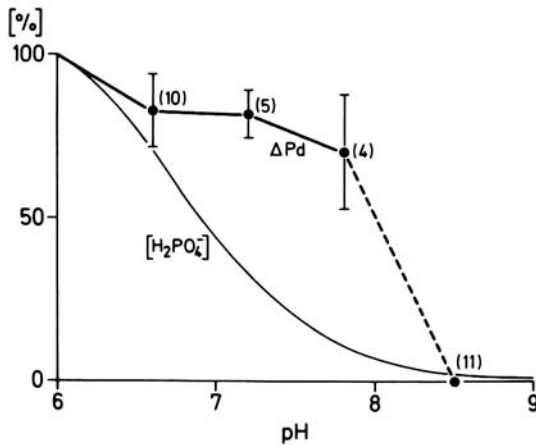


Figure 2. pH-dependence of the cell potential response to luminal application of 2mmolar P_i . Abscissa: pH, ordinate: ΔPd in percentage of the control value observed at pH 6.0. The symbols (●—●) represent mean values \pm SE with number of tubules studied in brackets. The faint line represents the luminal concentration of $H_2PO_4^-$, also expressed in percentage of its value at pH 6.0. Note that ΔPd falls more slowly than $[H_2PO_4^-]$

- a) In case we are dealing with a single transport system which does not discriminate between H_2PO_4^- and HPO_4^{--} we would have to postulate that the maximal transport capacity increased with increasing pH, and
- b) In case of two separate transport systems for H_2PO_4^- and HPO_4^{--} we would have to postulate that the H_2PO_4^- system exhibited saturation kinetics with a half saturation constant of the order of 0.1mmol/L.

The first possibility can be excluded for quantitative reasons: to explain a reduction of the potential response from 100 per cent (at pH 6.0) to 69.8 per cent (at pH 7.8), when the H_2PO_4^- concentration fell to 10.5 per cent of its value at pH 6.0, would require that the stoichiometry of Na^+ to H_2PO_4^- increased from 2:1 at pH 6.0 to 14:1 at pH 7.8, which is unlikely.

The possibility 2a agrees with experiments on brush border membrane vesicles, which suggested that a single P_i transport system is present, which accepts both H_2PO_4^- and HPO_4^{--} indiscriminately and whose maximal transport rate increases around five- to six-fold when pH increases from 6.0 to 7.8 [5, 9]. This compares well with the near seven-fold increase which we derive from our experiments for the same pH range, although the quantitative agreement at lower pH values is less perfect (see Table I).

TABLE I. pH-dependence of v_{max} for the model of indiscriminate absorption of H_2PO_4^- and HPO_4^{--}

pH	v_{max}
6.0	1.0
6.6	1.17
7.2	2.48
7.8	6.65

The change of v_{max} was calculated by dividing ΔPd by $[\text{H}_2\text{PO}_4^-]$ in Figure 2. Further observations with 4mmolar P_i indicate that the transport is practically saturated at the experimental concentration of 2mmol/L, so that affinity changes can be ruled out

The possibility 2b, however, cannot be definitely excluded at present as long as the concentration dependence of the potential response to varying H_2PO_4^- concentrations at constant luminal pH is not known.

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