Lack of a direct role for cyclic AMP in parathyrin action on phosphate reabsorption by the kidney

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Isolated chick kidney proximal tubule cells have been used in a study of the mechanism by which PTH inhibits its Na^+-dependent P_i transport in the kidney. Treatment with PTH inhibits P_i uptake by the cells by 13% and stimulates cyclic AMP production by 77%. Forskolin, a potent activator of adenyl cyclase, brought about an 11-fold stimulation of cyclic AMP production by the cells, but in contrast to PTH, the drug had no effect on Na^+-dependent P_i uptake. These results provide evidence that PTH action on phosphate transport is not mediated by cyclic AMP.

Kidney tubule *Parathyroid hormone* *Cyclic AMP* *Phosphate transport*

1. INTRODUCTION

The lumenal membrane of kidney proximal tubule cells contains a sodium-dependent phosphate carrier [1]. In vivo, PTH release induces phosphaturia and several studies have revealed that a change in the properties of the tubule transport system is likely to be the most important factor in the process by which the hormone increases phosphate excretion [2]. Studies conducted on whole animals and on isolated nephrons have shown that PTH stimulates the production of cyclic AMP by kidney tubules [3] leading to the belief that the cyclic nucleotide acts as a second messenger for PTH. By analogy with other systems, it is possible that PTH effects could be transmitted by the phosphorylation of sensitive proteins by cyclic AMP-dependent protein kinases. Phosphorylation of specific tubule membrane proteins in dogs has been demonstrated following PTH administration [4,5]. Attractive as this hypothesis may seem however, it has been difficult to provide evidence that cyclic AMP release and phosphaturia are releated as cause and effect [3].

To study the mechanism of PTH action we are using an isolated tubule cell preparation. This system contains the phosphate transporter and also retains metabolic competence and full hormone responsiveness. It provides a technically more convenient model for studying transport than isolated perfused kidneys and has the additional advantage that it represents a single cell type.

Abbreviation: PTH, parathyroid hormone

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2. EXPERIMENTAL

Proximal tubule cells were isolated following the digestion of chick kidney tissue by collagenase-hyaluronidase treatment [6]. The chicks were reared on a normal diet and used when 3–5 weeks old. Na^+-dependent uptake of P_i by the cells was determined at 37°C using $^{32}P_i$ [5]; 3′-5′-cyclic AMP was determined using reagents supplied by BDH Chemicals by a method that follows the protocol of the kit obtainable from Amersham International. Hormone responsiveness was tested by treating the cell suspension at 37°C with PTH (61–84 peptide fragment) for 6 min. In these incubations, approximately 2.5 mg cells were present

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per ml incubation and the hormone was used at a concentration of 1 USP unit per ml. At the end of the 6-min period, the cells were taken for measurement of the initial rate of P_i uptake and of cyclic AMP production. In some incubations, forskolin (Calbiochem) was included at 10 μmol/l instead of PTH. Under the conditions of these experiments, the rate of release of $^{14}CO_2$ from [U-^{14}C]glucose was linear for at least 1 h.

3. RESULTS AND DISCUSSION

At 1 unit per ml, incubation with PTH for 6 min decreased the initial rate of P_i uptake by 13% (fig.1). This effect of PTH was dose-dependent and a half-maximal response was obtained at hormone levels of approx. 0.2 units/mg cells [7]. The hormone treatment stimulated cellular cyclic AMP production by 77% (fig.2). The changes in uptake

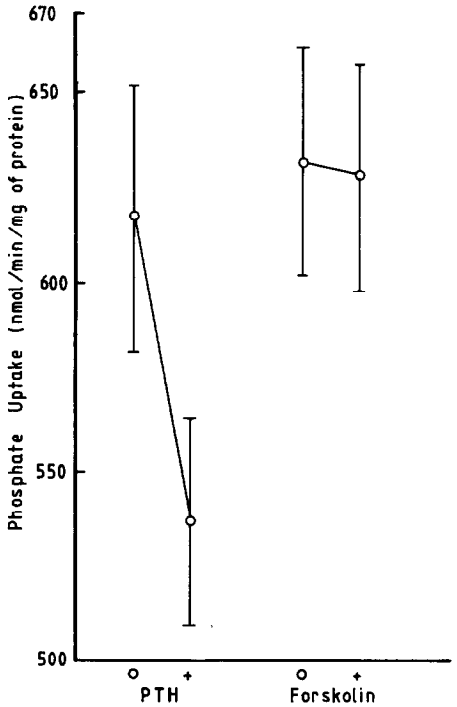

Fig.1. Na^+-dependent P_i uptake by chick kidney proximal tubule cells. The cells were incubated for 6 min at 37°C with or without PTH at 1 USP unit per ml. Alternatively, the cells were incubated with or without 10 μmol/l forskolin. The points shown represent the mean ± SE for 17 separate determinations (PTH) or for 15 determinations (forskolin).

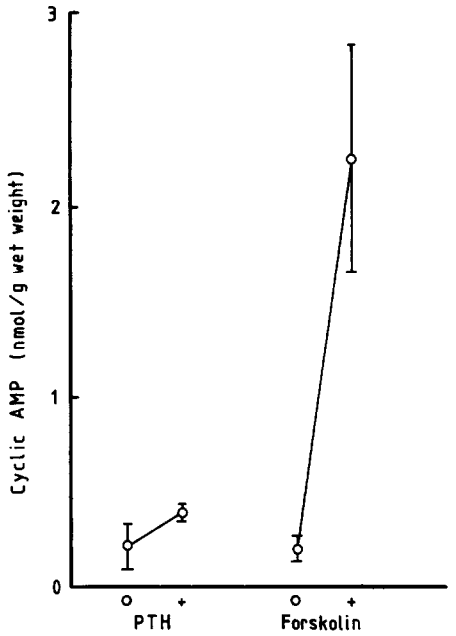

Fig.2. Cyclic AMP production by chick kidney proximal tubule cells. The cells were incubated with PTH or forskolin (see fig.1) before determination of cyclic AMP. The points shown represent the mean ± SE of 4 separate determinations.

and cyclic nucleotide production are comparable with those reported by workers using intact tissue preparations and similar hormone doses [8,9].

The diterpene forskolin is a powerful activator of adenyl cyclase in intact cell and membrane preparations and has been shown to act potently on rat and chick renal adenyl cyclase [10]. We attempted to mimic the action of PTH on chick kidney cells by using forskolin. As shown in fig.2, the drug brings about a very large stimulation (approx. 11-fold) of cyclic AMP production. Remarkably however, no significant inhibition of P_i uptake by the cells accompanies forskolin treatment (fig.1). The degree of stimulation of cyclic AMP formation seen with the isolated cells is very similar to that reported for experiments conducted on kidney slices from 8-week-old chicks [9].

These results clearly show that while the PTH-induced inhibition of P_i uptake by proximal tubule cells is accompanied by some stimulation of cyclic AMP production, the events are unlikely to be directly related. PTH has a negligible effect on cyclic AMP compared with forskolin, yet the

relatively massive formation of the nucleotide produced by the drug has no apparent action on P_i uptake. If phosphorylation of specific membrane proteins is part of the mechanism by which the cell transmits information from the occupied PTH receptor to the tubule phosphate transport system [4,5], consideration must be given to the possibility that the phosphorylations are brought about by protein kinases that do not require cyclic AMP. In this regard, much attention is now being paid to the role of protein kinase C in mediating hormone action [11,12].

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