Vol. 84, No. 2, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
September 29, 1978 Pages 396-402

PARATHYROID HORMONE INDUCED STIMULATION OF CALCIUM UPTAKE BY RENAL MICROSOMES

E. Harada*, S.G. Laychock, and R.P. Rubin**

Department of Pharmacology
Medical College of Virginia
Richmond, Virginia 23298 USA

Received August 11, 1978

SUMMARY

Cortical and papillary microsomes prepared from feline kidneys perfused with parathyroid hormone (PTH) showed an enhanced ability to accumulate calcium (Ca^{+2}). PTH was unable to stimulate Ca^{+2} uptake into microsomes prepared from outer medulla. These data suggest that renal microsomes may be a valid model system for studying the action of PTH on Ca^{+2} transport in the kidney.

INTRODUCTION

Parathyroid hormone (PTH) exerts its hypercalcemic action in part by decreasing the renal clearance of calcium (Ca^{+2}) (1,2). Although the site of action of this hormone within the nephron remains to be clearly defined, an effect of PTH on the proximal tubule might be inferred from early findings that a significant proportion of the cellular transfer of Ca^{+2} occurs in the proximal portion of the nephron (3-5). Nevertheless, clearance and micropuncture studies suggest that PTH may act at several sites, particularly the distal tubules (1,2,6).

Since microsomal membranes isolated from the renal cortex exhibit an ATP-dependent Ca^{+2} uptake system (7,8), this model could provide more information relating to the PTH-regulated mechanism for Ca^{+2} transport. Accordingly, a study of the effects of PTH on Ca^{+2} uptake by renal membrane fractions was undertaken in order to characterize further the site of action of PTH in the kidney.

*Present address: Department of Physiology, School of Veterinary Medicine, Hokkaido University, Sapporo 060, Japan.
**To whom all correspondence should be sent.

0006-291X/78/0842-0396$01.00/0

MATERIALS AND METHODS

Cats (2-4 kg) were anesthetized with pentobarbital and both kidneys were prepared for perfusion as previously described (9). The kidneys were removed from the animal, placed in a plexiglass chamber, and perfused for 100 min with Locke solution. In certain experiments one of the paired kidneys was perfused with PTH during the final 20 min of perfusion. At the end of the perfusion period the kidneys were decapsulated and beginning at the capsular margin were sectioned inward to isolate 5 mm of cortex and 5 mm outer medulla, and the inner medulla (papilla). Each renal zone was minced separately and 1.0 gm of tissue was homogenized at 4°C in 0.25 M sucrose using a Teflon pestle. The homogenate was filtered through nylon mesh (100 μm) and sequentially centrifuged at 4°C at 1475g (10 min), 15000g (10 min), and 27000g (30 min). The 27000g pellet (microsomal fraction) was resuspended in 0.25 M sucrose to attain a final protein concentration of 0.6 mg/ml. Ca^{+2} accumulation by 0.2 or 0.4 ml aliquots of renal microsomes was determined by Millipore filtration as previously described (10). The incubation medium had the following composition (mM): KCl, 100; imidazole-histidine buffer (pH 6.8), 30; Na_2ATP, 5; $MgCl_2$, 5; Ammonium oxalate, 5; sodium azide, 5; and $CaCl_2$, 0.01. $^{45}CaCl_2$ was present in a final concentration of 0.1 μCi/ml. Protein concentrations of cortical, medullary, and papillary microsomes were determined by the method of Lowry et al. (11), and equal protein concentrations were used in the assay. Ca^{+2} accumulation is expressed as nmol Ca^{+2}/mg protein or in the case of hormone-treated preparations as a percent of values obtained from the control kidney of the same animal.

Bovine PTH (600 PTU units/mg) was obtained from Calbiochem. A stock solution was prepared by dissolving the material in 0.1% sodium acetate containing 0.1% govine serum albumin and stored at -10°C. Immediately prior to perfusion, an aliquot (60-250 μl) of the thawed solution was added to 140 ml of Locke solution. This addition did not alter the pH of the perfusion medium. Angiotensin, a generous gift from Ciba Pharmaceuticals, was dissolved in saline prior to use.

Statistical evaluation of results was based upon analysis of variance (split plot analysis) or paired Student t test. Analysis of variance was used to assess the significance of differences in Ca^{+2} uptake over time by microsomes from control and PTH-treated kidneys. The paired t test was applied to assess the significance of differences at one particular time point by comparing Ca^{+2} uptake by microsomes from a hormone-treated kidney as a percent of the uptake by microsomes from the control kidney of the same animal.

RESULTS

Figure 1 illustrates the Ca^{+2} accumulating activity of microsomal fractions prepared from cortex, medulla, and papilla of the cat kidney. Cortical microsomes had the highest Ca^{+2} accumulating activity; after 60 min, Ca^{+2} uptake by cortical microsomes was approximately 2 and 3 times greater than the Ca^{+2} uptake by microsomes from medulla and papilla, respectively. The Ca^{+2} uptake by the 27000g fraction was almost completely ATP dependent. Thus, in the absence of ATP, Ca^{+2} uptake by cortical,

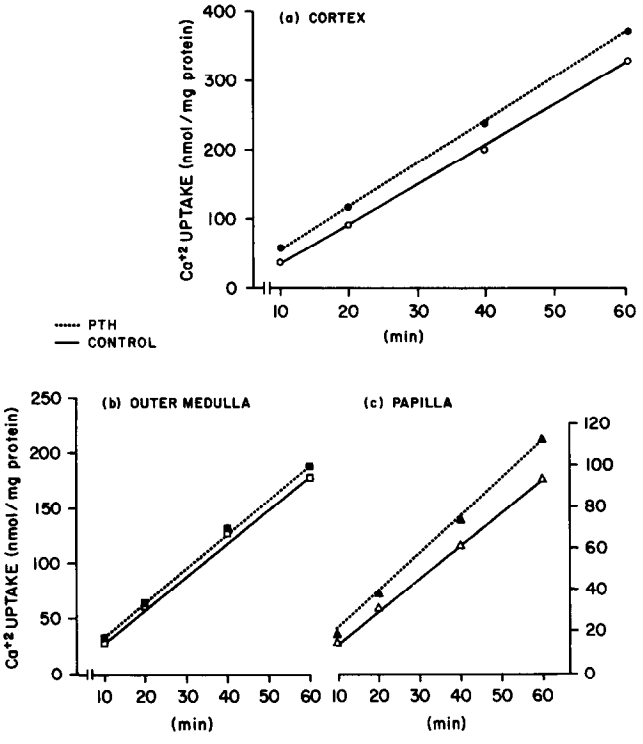

Fig. 1. Ca^{+2} accumulation by cat renal microsomes isolated from (a) cortex, (b) medulla and (c) papilla. Paired kidneys were perfused for 20 min in the presence or absence of PTH (25-100 mU/ml). Microsomes prepared from each area of the kidney by centrifugation at 27000g were incubated at 37°C with complete assay medium for varying periods of time. Results represent total Ca^{+2} accumulation, including ATP-independent Ca^{+2} binding in membranes and/or filters, and are expressed as mean values for 4 separate experiments. For additional details see Results.

medullary, and papillary microsomes after 60 min was 9.5 (±0.1), 6.7 (±0.2), and 5.4 (±0.7) nmol/mg protein, respectively, which is only 4-6% of the uptake after 60 min obtained in the presence of ATP (see Fig. 1). Ca^{+2} uptake was also abolished by removing the calcium precipitant, ammonium oxalate, from the incubation medium (data not shown). On the other hand, when the mitochondrial inhibitor, sodium azide, was removed from the incubation medium, Ca^{+2} accumulation by cortical microsomes was 100.2% of that obtained in the presence of azide. The lack of effect of azide denotes the absence of mitochondria; this was

Table 1

Effect of PTH on Ca^{+2} accumulation by microsomes from renal cortex, outer medulla and papilla

Expt.		Cortex Ca^{+2} uptake nmol/mg protein	% of Control	Outer Medulla Ca^{+2} uptake nmol/mg protein	% of Control	Papilla Ca^{+2} uptake nmol/mg protein	% of Control
1	Control	118	112	51	96	24	119
	PTH	132		49		29	
2	Control	84	134	93	94	38	129
	PTH	112		87		48	
3	Control	120	137	73	105	28	132
	PTH	164		77		37	
4	Control	45	128	24	93	29	117
	PTH	58		22		34	
		Mean(±SEM)	128(±6)*	Mean(±SEM)	97(±3)**	Mean(±SEM)	124(±4)***
5	Control	114	83	40	103	36	97
	Angiotensin	94		41		35	

Paired kidneys were perfused with Locke solution for 20 min in the presence or absence of PTH (25-100 mU/ml) or angiotensin (50 ng/ml). The kidneys were sectioned into 3 regions and microsomal vesicles prepared (see Methods). Ca^{+2} uptake was determined after a 20 min period of incubation.
*$p<0.05$
**$p>0.5$
***$p<0.01$

confirmed by electron microscopic analysis, which showed that the cortical microsomes contained no intact mitochondria but consisted almost entirely of rounded vesicles and some tubular structures.

The addition of PTH to the perfused kidney stimulated Ca^{+2} accumulation in cortical and papillary microsomes (Fig. 1a,c; Table 1). No effect of PTH was observed on medullary microsomes (Fig. 1b; Table 1). Although a complete dose-response relation was not delineated, PTH in the concentration range of 25-100 mU/ml activated Ca^{+2} accumulation to a similar degree. Analysis of variance demonstrated that the Ca^{+2} uptake curves generated from control and hormone-treated kidneys were parallel in each case ($P<0.0001$) (Fig. 1b,c). While this same statistical procedure showed that the stimulant effect of PTH on Ca^{+2} accumulation by papillary microsomes was highly significant ($P<0.01$), the stimulant effect of PTH on cortical microsomes was significant only at

the 0.085 level. A more acceptable significance level ($P<0.05$) was obtained when Ca^{+2} uptake by cortical microsomes from PTH-treated kidneys was compared with Ca^{+2} uptake by microsomes from the control kidney of the same animal by paired Student t test (Table 1). Although the effect of PTH on papillary microsomes still attained a higher level of significance ($P<0.01$), PTH activated Ca^{+2} uptake by cortical and papillary microsomes to approximately the same extent. After 20 min, PTH stimulated Ca^{+2} uptake in cortical and papillary microsomes by 28 and 24%, respectively (Table 1). In contrast to the stimulant action of PTH, perfusion with angiotensin (50 ng/ml) for 20 min failed to activate Ca^{+2} uptake by microsomes prepared from cortex, outer medulla, or papilla (Table 1).

DISCUSSION

The present study has demonstrated that cortical and papillary microsomes prepared from kidneys perfused with PTH manifest an enhanced ability to accumulate Ca^{+2}. The specificity of the stimulatory effect is evidenced by the inability of PTH to stimulate Ca^{+2} uptake into microsomes prepared from outer medulla. Moreover, angiotensin, another peptide hormone, failed to stimulate Ca^{+2} uptake in any of the three regions of the kidney. Although PTH is capable of stimulating Ca^{+2} transport in isolated mitochondria (12), the ability of PTH to increase Ca^{+2} accumulation by vesicle membranes which were devoid of mitochondria indicates an action of the hormone on non-mitochondrial membrane systems. However, it is not possible at present to determine whether vesicles derived from plasma membrane or endoplasmic reticulum were responsible for the PTH-induced increase in Ca^{+2} uptake. In this regard, Borle and Uchikawa (13) have recently provided evidence that PTH stimulates Ca^{+2} transport in isolated kidney cells, although they, too, were unable to ascertain whether a primary site of action was the plasma membrane or an intracellular organelle.

Any conclusions gleaned from the present study must be tempered by the awareness that the microsomes derived from each of the three regions of the kidney were heterogeneous in that the vesicle membranes were in all probability derived from several different cell types. Despite this reservation, the renal microsomes may be a valid model system for studying the action of PTH on Ca^{+2} transport in the kidney. Thus, Ca^{+2} uptake was highest in microsomes prepared from cortex which is rich in proximal tubules where the bulk of active Ca^{+2} transport takes place (3,4). Additionally, Ca^{+2} transport also occurs to a more limited extent throughout the remainder of the nephron and collecting ducts (3), which parallels the lesser ability of medullary and papillary microsomes to accumulate Ca^{+2}. Despite the relatively large amount of Ca^{+2} which is reabsorbed at the proximal tubule, there are several sources of evidence suggesting that the distal convoluted tubule and/or collecting duct may be the site at which PTH enhances Ca^{+2} reabsorption (6,14). Although the present data are more consistent with a dual site of action of PTH, it is of interest to note that while the effect of PTH on Ca^{+2} uptake by cortical and papillary microsomes was of similar magnitude, the effect on papillary microsomes had a greater degree of statistical significance. Thus, our data argue in favor of a fundamental action of PTH at a more distal site - more specifically the collecting duct since the papillae are rich in these structures.

ACKNOWLEDGMENTS

This work was supported by United States Public Health Service Grant AM-18066. EH was a recipient of an A.D. Williams Visiting Scholar Fellowship during part of his stay at the Medical College of Virginia.

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