Biochemical Pharmacology, Vol. 25, pp. 219–220. Pergamon Press, 1976. Printed in Great Britain.

Stimulation of *p*-aminohippuric acid transport in rabbit kidney cortex by parathyroid hormone and adenosine 3′,5′-cyclic monophosphate

(*Received* 17 *February* 1975; *accepted* 25 *April* 1975)

Parathyroid hormone (PTH) affects the renal handling of not only electrolytes but also of sugars and amino acids [1]. There appear to be substantial differences in the way in which PTH affects the transport of non-electrolytes. Weiss *et al.* [2] reported that PTH and dibutyryl-cyclic AMP (DB-cAMP) stimulate amino acid uptake by slices of rat kidney cortex. This effect requires preincubation with DB-cAMP for over 90 min and is blocked by inhibitors of protein synthesis (cycloheximide and puromycin). Reyholds and Segal [3] have shown that uptake of α-methyl-D-glucoside by slices of rabbit kidney cortex is increased by PTH and DB-cAMP. This stimulation requires pretreatment with DB-cAMP but is not blocked by cycloheximide. Both studies suggest that PTH and DB-cAMP somehow increase the activity of the transport process normally involved in reabsorbing glucose and amino acids from the lumen of the proximal tubule into the tubule cell across the lumenal brush border. On the other hand, Short *et al.* [4] have shown that in humans infusion of PTH has an immediate effect of decreasing tubular reabsorption of amino acids. It is not yet clear whether the conflicting results of these studies represent species differences or methodological differences (i.e. slices as opposed to clearance techniques *in vivo*).

We proposed to determine if PTH and cyclic AMP might also influence renal secretory transport by studying uptake of the organic anion *p*-aminohippuric acid (PAH) which is actively transported from renal peritubular capillaries into the proximal tubule cell [5]. We prepared suspensions of separated renal cortical tubules by a modification of the method of Nagata and Rasmussen [6]. New Zealand white rabbits of either sex were killed by injecting air into an ear vein. The kidneys were immediately removed and placed in a buffer containing 10 mM sodium phosphate (mono- and di-basic adjusted to a final pH of 7·40), 120 mM NaCl, 16·2 mM KCl, 10 mM Na acetate, 1·2 mM $MgSO_4$, and 1·0 mM $CaCl_2$ (standard buffer). The cortex of each kidney was removed by careful dissection and put through a tissue press (1·5 mm pore size). Each kidney was digested at 37° for 1 hr in 20 ml standard buffer containing 20 mg collagenase (Sigma). The tubules were then dispersed with a wide tipped pipette and filtered through a single layer of nylon stocking. They were washed three times in standard buffer by centrifugation at 800 rev/min for 45 sec. The resulting tubule suspension from each kidney was resuspended in approximately 3 ml buffer to give a tubule protein concentration of 50–60 mg/ml.

In our uptake experiments, 100 μl of tubule suspension was added to a polypropylene test tube containing 1 ml standard buffer and 10 mg/ml of rabbit serum albumin. The tube was oxygenated for 30 sec, capped tightly, and placed on a shaking water bath at 37° for the desired preincubation period. During preincubation, some tubes contained 10 μg/ml of purified bovine parathyroid hormone (gift of Dr. H. Rasmussen, University of Pennsylvania) or 10^{-3} or 10^{-4} M cAMP (CalBiochem). In some experiments, we also added 1 mM cycloheximide (Sigma). At the end of the preincubation period, we added 20 μl buffer containing PAH [glycyl-l-^{14}C] (New England Nuclear, 32·4 mCi/m-mole) to a final concentration of 8 μM. After 15 min, a 0·5-ml aliquot was removed from each tube, filtered under vacuum through an 8 μm pore size filter (Nucleopore) and washed twice with 4 ml buffer. The filter was placed in a vial containing 10 ml Aquasol (New England Nuclear) and counted in a liquid scintillation spectrometer. Data are reported as cpm/mg of tubule protein and were corrected for non-specific binding of PAH to renal tissue.

Table 1 shows the effect on PAH uptake of preincubating renal cortical tubules for 130 min with cAMP or PTH prior to the addition of PAH. cAMP at 10^{-3} M consistently inhibited PAH uptake by more than 50 per cent, probably by a competitive mechanism, as suggested by Podevin and Boumendil-Podevin [7]. In other experiments not reported here, DB-cAMP inhibited PAH uptake by an even greater amount. However, cAMP at a concentration of 10^{-4} M and PTH increased the rate of PAH uptake significantly.

Figure 1 shows the time course of the stimulatory effect of PTH on PAH uptake. PTH apparently begins to exert an effect within the first 15 min, and the magnitude of the effect increases linearly with time.

As shown in Fig. 2, 1 mM cycloheximide partially reduces the stimulatory effect of PTH on PAH uptake, but also reduces PAH uptake by itself.

These experiments clearly show that PTH and cAMP (at 10^{-4} M) increase the rate of uptake of PAH by renal tubules. A recent report by Costanzo and Weiner [8] suggested that PTH may increase the secretion of chlorothia-

Table 1. PAH uptake by renal tubules after preincubation with cAMP or PTH

Agent and dose	Number of experiments	Mean cpm + S. E. M. Control	Experimental	% Change	P*
cAMP (10^{-3}M)	7	230·7 ± 34·1	105·3 ± 12·0	−54	< 0·01
cAMP (10^{-4} M)	13	244·3 ± 30·1	301·8 ± 37·8	+23	< 0·01
PTH (10 μg/ml)	11	292·3 ± 37·6	323·0 ± 41·7	+11	< 0·01

* Using Student's *t*-test for paired data.

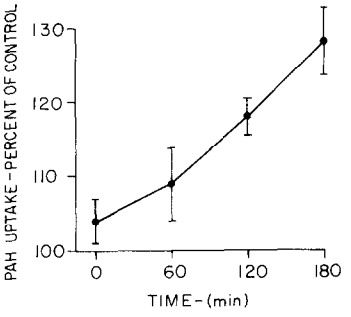

Fig. 1. Time course of the effect of PTH on PAH uptake. All tubules were incubated for 180 min and PTH was added at 0, 60, 120 or 180 min prior to the addition of PAH. Data represent mean ± S. E. M. of three experiments run in quadruplicate.

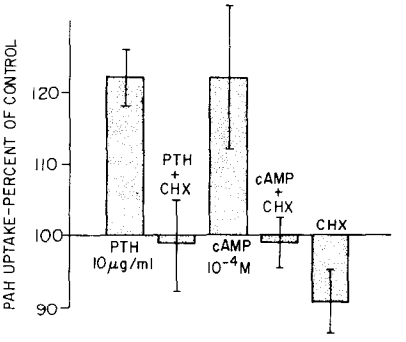

Fig. 2. Effect of cycloheximide (CHX) on the stimulation of PAH uptake by PTH and cAMP. Tubules were incubated as described for 130 min with either 10 μg/ml of PTH or 10^{-4} M cAMP. Some tubes also contained 1 mM cycloheximide. Note that cycloheximide has only partially reduced the stimulation of PAH uptake by PTH and cAMP because the PAH uptake in the tubules containing cycloheximide alone was reduced to 90 per cent of the control value. Data show mean ± S. E. M. of three experiments run in quadruplicate.

zide, another organic anion. Thus, it is likely that the effect shown in the present experiments results from stimulation of the renal transport system for organic anions. However, our experiments do not explain the mechanism of the stimulation of transport. While stimulation of PAH uptake is reduced by cycloheximide, there does not appear to be a lag phase during which stimulation does not occur, as seen in the stimulation of glucose and amino acid transport by DB-cAMP. The finding of such a lag phase has often been used to support a hypothesis that an effect is mediated via synthesis of new protein. Therefore, we cannot say at this point whether or not synthesis of transport protein might be involved. Furthermore, we cannot determine whether or not the effect of PTH is a direct one or is secondary to increased intracellular production of cAMP. However, the latter possibility is probably a reasonable working hypothesis until the exact mechanism of the stimulation of transport can be elucidated.

Acknowledgements— We thank Naomi Kaji for her excellent technical assistance. This work was supported by NIH General Research Support Grant 5S01-RR-05468, and by grants from the Arthritis Foundation (Southern California Chapter) and the Kidney Foundation and Diabetes Association of Southern California.

* Senior Investigator, American Heart Association, Greater Los Angeles affiliates. Present address: Division of Nephrology Department of Medicine, University of Southern California, Los Angeles, Calif.

Department of Pharmacology, UCLA School of Medicine, Los Angeles, Calif. 90024, *and Divisions of Medicine, Cedars-Sinai Medical Center, Los Angeles, Calif.* 90048, *U.S.A.*

IAN KIPPEN
KIYOSHI KUROKAWA*
JAMES R. KLINENBERG

REFERENCES

1. G. D. Aurbach and D. A. Heath, *Kidney Int.* **6**, 331 (1974).
2. I. W. Weiss, K. Morgan and J. M. Phang, *J. biol. Chem.* **247**, 760 (1972).
3. R. Reynolds and S. Segal, *Am. J. Physiol.* **226**, 791 (1974).
4. E. M. Short, L. J. Elsas and L. E. Rosenberg, *Metabolism* **23**, 715 (1974).
5. R. J. Cross and J. V. Taggart, *Am. J. Physiol.* **161**, 181 (1950).
6. N. Nagata and H. Rasmussen, *Biochim. biophys. Acta* **215**, 1 (1970).
7. R. A. Podevin and E. F. Boumendil-Podevin, *Biochim. biophys. Acta* **375**, 106 (1975).
8. L. S. Costanzo and I. M. Weiner, *J. clin. Invest.* **54**, 628 (1974).

Biochemical Pharmacology, Vol. 25, pp. 220–221. Pergamon Press, 1976. Printed in Great Britain.

Reduced incorporation of [^{3}H]leucine into cerebral proteins after long-term ethanol treatment

(*Received* 8 *January* 1975; *accepted* 2 *September* 1975)

Results concerning effects of long-term ethanol consumption on the incorporation of labelled amino acids into brain proteins of intact animals are scarce and conflicting [1,2]. In these studies ethanol solutions were given to the experimental groups instead of water. This procedure provides more 'empty' calories to the experimental animals than to the control animals. The subsequent reduced intake of protein or other essential nutrients by treated rats could thus be responsible for the apparent effect of ethanol on protein metabolism. Such effects could also vary among different experiments if the composition of the basic diet varied. The question was raised: whether ethanol intake could influence cerebral protein synthesis, independently of nutrition. In our experiments ehtanol was given to replace lipids isocalorically in a way which did not influence the daily intake of minerals, vitamins, protein or other essential nutrients.