Hormonal Control of the Renal Conversion of 25-Hydroxycholecalciferol to 1,25-Dihydroxycholecalciferol

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ABSTRACT Isolated renal tubules from vitamin D-deficient chicks catalyse the in vitro conversion of 25-hydroxycholecalciferol to 1,25-dihydroxycholecalciferol. This conversion is stimulated by 5×10^{-10} M bovine parathyroid hormone, or by 10^{-6} M cyclic AMP. It is inhibited by 10^{-9} M porcine calcitonin. It is concluded that these hormonal controls of the synthesis of the renal hormone 1,25-dihydroxycholecalciferol are of particular physiological significance in coordinating the activities of the various organs involved in extracellular calcium homeostasis.

INTRODUCTION

It has been established that before exerting its biological effects vitamin D is metabolized to more polar, biologically active metabolites (1). The first of these, 25-hydroxycholecalciferol (25[OH]D_3)[1] was shown to be produced by a hepatic hydroxylation system (2). The second, which is produced in the kidney (3), has been identified as 1,25-dihydroxycholecalciferol (1,25[OH]$_2D_3$) (4) and shown to be the metabolite responsible for the increase in intestinal calcium transport observed after vitamin D administration to D-deficient animals (4, 6).

Boyle, Gray, and DeLuca have shown that when the plasma calcium concentration in D-deficient rats is low, the rate of conversion of 25(OH)D_3 to 1,25(OH)$_2D_3$ in vivo (as measured by the amount of 1,25[OH]$_2D_3$ isolated from the intestinal mucosa) is increased, but the conversion to 21,25(OH)D_3 was decreased (6). Conversely, a high plasma calcium concentration was associated with a low rate of 25(OH)D_3 to 1,25(OH)$_2D_3$ conversion, but a high rate of 25(OH)D_3 to 21,25-(OH)$_2D_3$ conversion. These data were interpreted as evidence for a direct effect of plasma calcium ion concentration upon the renal metabolism of 25(OH)D_3. However, subsequent work by Shain (7) showed that isolated renal tubules from kidneys of D-deficient chicks were able to synthesize 1,25(OH)$_2D_3$, but that changes in extracellular calcium ion concentration in vitro had little effect upon the rate of conversion of 25(OH)D_3 to 1,25(OH)$_2D_3$. These results were inconsistent with the tentative model of control suggested by Boyle, et al. (6).

It remained a distinct possibility, as Boyle and coworkers (6) recognized, that the renal metabolism of 25(OH)D_3 was not directly controlled by changes in plasma calcium concentration but indirectly via the effects of changes in plasma calcium concentrations upon the rates of secretion of parathyroid hormone (PTH) and calcitonin (CT) (8). An increase in PTH secretion is seen when plasma calcium concentration falls. An increase in CT secretion is seen when plasma calcium concentration rises. If hormonal, rather than direct ionic control of the renal metabolism of 25(OH)D_3 to 1,25(OH)$_2D_3$ were responsible for the changes in rates of 1,25(OH)$_2D_3$ synthesis, then one would predict that in vitro the addition of PTH would stimulate the conversion of 25(OH)D_3 to 1,25(OH)$_2D_3$ by isolated renal tubules and CT would inhibit this conversion. We wish to report a verification of these predictions.

METHODS

Isolated chick renal tubules were prepared by a technique previously described employing enzymatic digestion of minced renal tissue with hyaluronidase and collagenase (7, 9). After preparation in a modified Krebs-Ringer bicarbonate buffer, the tubules were washed and diluted to a concentration of 10 mg tubule protein per ml. 1-ml portions from this prepa-

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[1] *Abbreviations used in this paper:* CT, calcitonin; KRB, Krebs-Ringer bicarbonate buffer; 1,25(OH)$_2D_3$, 1,25-dihydroxycholecalciferol; 25(OH)D_3, 25-hydroxycholecalciferol; POPOP, 1,4-bis[2-(5-phenyloxazolyl)]benzene; PPO, 2,5-diphenyloxazole; PTH, parathyroid hormone.

ration were placed in each incubator flask to which was added the appropriate hormone or carrier. The reaction was started with the addition of 0.3 μg of [26,27-$^3H_2$] 25-hydroxyvitamin D_3 (New England Nuclear Corp., Boston, Mass., specific activity 315 mCi/mmole). Incubations were carried out in siliconized 25-ml flasks for 15 or 30 min in KRB containing 2.0% bovine serum albumin, 1.0 mM $CaCl_2$ 2 mM Na pyruvate, and 2 mM glucose at pH 7.4 and 37°C with 95% O_2–5% CO_2 as the gas phase. The reaction was stopped by the addition of 5 ml of ice-cold 95% ethanol. The cells were collected by centrifugation, the supernate decanted, and the cell pellet then extracted three times with 95% ethanol, two times with $CHCl_3$: ethanol (2:1), and once with diethyl ether. The combined extracts were taken to dryness under N_2, taken up in a small volume of ethyl acetate, and applied to a thin layer of Silica Gel G (0.5 mm in thickness; Brinkmann Instruments, Inc., Westbury, N. Y.), before development with 100% ethyl acetate in complete darkness for 40 min. Sixteen 1 cm strips of the Silica Gel were scraped from the plate into a scintillation vial containing 0.3 ml H_2O, and 10 ml of a counting solution consisting of the toluene solution of PPO-POPOP mixed with methyl cellusolve in the ratio of 100:60 (v/v). Final recovery of radioactivity added to the incubation was 75–80%; counting efficiency was approximately 20%. All counting was performed in a Packard model 3320 scintillation counter (Packard Instrument Co., Inc., Downers Grove, Ill.). All samples were corrected for quenching by the channels ratio procedure. Identification of 25-hydroxy- (R_f = 0.63) and 1,25-dihydroxycholecalciferol (R_f = 0.40) was verified by previously described methods, which included counter-current distribution, Sephadex LH-20 (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) chromatography, sensitivity to periodate, and examination of the biological activity of the isolated product (7). The data were first expressed in disintegrations per minute, and then converted to nanograms of $1,25(OH)_2D_3$ produced. Protein was determined by the method of Lowry, Rosebrough, Fair, and Randall (10). Parathyroid hormone was prepared by the method of Hawker, Glass, and Rasmussen (11), and calcitonin was a gift from Dr. Claude Arnaud. The hyaluronidase and collagenase and cyclic 3'5'-adenosine monophosphate were purchased from the Sigma Chemical Company, St. Louis, Mo.

RESULTS AND DISCUSSION

The rate of $1,25(OH)_2D_3$ production was linear for at least 1 hr when 300 ng of $25(OH)D_3$ was incubated with renal tubules equivalent to approximately 10 mg of protein. In 30 min, approximately 40% of the added $25(OH)D_3$ was converted to $1,25(OH)_2D_3$ and approximately 55% of the $25(OH)D_3$ had disappeared. To examine the effects of hormones and nucleotides, 15-min incubations were performed.

The effects of PTH, CT, and cAMP upon the conversion of $25(OH)D_3$ to $1,25(OH)_2D_3$ are summarized in Table I. The addition of 0.5 ng PTH per ml consistently caused a slight decrease ($P < 0.1$) in the amount of $1,25(OH)_2D_3$ produced. A concentration of 1.0 ng/ml had no effect, but doses between 5.0 and 50.0 ng/ml stimulated $1,25(OH)_2D_3$ production. A 10-fold higher concentration (500 ng/ml) had no effect upon $1,25(OH)_2D_3$ production.

The other known effect of PTH upon renal metabolism is that of activating adenyl cyclase (12). Because of this fact, the effect of exogenous cyclic AMP upon $1,25(OH)_2D_3$ production was examined. The addition of 10^{-6} to 10^{-5} M cAMP caused a significant increase in

TABLE I

*Effect of Parathyroid Hormone, Calcitonin, and cAMP upon the In Vitro Synthesis of $1,25(OH)_2D_3$ from $25(OH)D_3$ in Isolated Chick Renal Tubules**

	PTH, ng/ml						
	0.0	0.5	1.0	5.0	10.0	50.0	500.0
$1,25(OH)_2D_3$, *ng/30 min per protein*	7.6±0.9	6.8±0.8	7.3±0.9	*12.5±1.8*	*14.0±2.1*	*13.6±2.2*	7.0±1.3
	CT, ng/ml						
	0.0	0.5	1.0	5.0	10.0	50.0	500.0
$1,25(OH)_2D_3$	7.6±0.9	7.4±1.1	6.6±0.9	*3.2±0.7*	*3.6±0.6*	*5.0±0.8*	*5.6±1.0*
			CT, 5 ng/ml + PTH				
			1.0	5.0	10.0		
$1,25(OH)_2D_3$			4.1±0.9	*11.1±1.8*	*12.9±1.9*		
	cAMP, M						
	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}		
$1,25(OH)_2D_3$	7.4±0.6	*9.1±0.8*	*11.0±1.3*	7.3±0.8	6.9±1.1		

* Those values in italics were significantly different ($P < 0.05$) from appropriate control values. All values were the mean ±SEM obtained from four separate experiments done for 15-min incubations.

$1,25(OH)_2D_3$ production, but 10^{-4} M or greater cAMP had no effect (Table I). Thus, in the case of both PTH and exogenous cAMP, there was biphasic response with stimulation of $1,25\ (OH)_2D_3$ production at low concentrations, but no stimulation at very high concentrations.

In contrast to PTH and cAMP, CT inhibited $1,25(OH)_2D_3$ production (Table I). The maximal effect was observed at a CT concentration of 5.0–10 ng/ml. 10- to 100-fold higher concentrations were less effective but still produced a significant inhibition. Also, when optimal doses of both PTH (10 ng/ml) and CT (5 ng/ml) were added simultaneously (Table I), CT did not block the effect of PTH. However, when the concentration of PTH was 1.0 ng/ml, CT caused a normal inhibition of $1,25(OH)_2D_3$ synthesis. These results mean that at high PTH (75.0 ng/ml) and normal or low CT (< 5.0 ng/ml) concentrations, the effect of PTH is seen, but at low PTH (< 1.0 ng/ml) and normal CT (5.0 ng/ml) concentrations, the effect of CT is seen. Thus, under physiological circumstances, in which one goes from a state of high CT and low PTH plasma concentrations, e.g. hypercalcemia, to one of low CT and high PTH, e.g. hypocalcemia, a 4- to 5-fold increase in the rate of conversion of $25(OH)D_3$ to $1,25(OH)_2D_3$ in the kidney could take place.

In view of these results, it seems likely that the relationship between the plasma calcium content and the in vivo production of $1,25(OH)_2D_3$ reported by Boyle, et al. (6), is related to the effect of plasma calcium concentrations upon the rates of PTH and CT secretion rather than a direct effect of plasma calcium concentration upon the renal conversion of $25(OH)D_3$ to $1,25(OH)_2D_3$.

The present results may account for the fact that PTH given in vivo increases the intestinal transport of calcium (13), but is without effect when added to intestinal mucosal sacs in vitro. Furthermore, although the time-course of the effects of the hormone upon both bone and kidney are rapid the effect upon intestinal calcium transport takes several hours to develop (3). These results are readily explained, if the effect of PTH upon intestinal calcium transport is not mediated by a direct action of the hormone upon this tissue but indirectly by the rate of synthesis of the renal hormone $1,25(OH)_2D_3$. If in addition, as seems likely (14), $1,25(OH)_2D_3$ is the biologically-active form of vitamin D in bone, and if, as also seems likely, this metabolite potentiates the action of PTH upon bone resorption, it becomes evident that the hormonal control of renal $1,25(OH)_2D_3$ production introduces an additional very exquisite control of calcium homeostasis, and is a means of coordinating the metabolic functions of the four organs, bone, intestine, kidney, and parathyroid gland, most directly involved in mammalian calcium homeostasis. In other words, PTH serves as a trophic hormone regulating the synthesis of the renal hormone, $1,25(OH)_2D_3$, and this hormone in turn regulates the function of other organs involved in calcium homeostasis. It is thus possible to define a type of hormonal cascade in the control of extracellular calcium homeostasis.

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REFERENCES

1. DeLuca, H. F. 1971. The role of vitamin D and its relationship to parathyroid hormone and calcitonin. *Recent Prog. Horm. Res.* **27**: 479.
2. Ponchon, G., A. L. Kennan, and H. F. DeLuca. 1969. Activation of vitamin D by the liver. *J. Clin. Invest.* **48**: 2032.
3. Fraser, D. R., and E. Kodicek. 1970. Unique biosynthesis by kidney of a biologically active vitamin D metabolite. *Nature (Lond.)*. **228**: 764.
4. Holick, M. F., H. K. Schnoes, H. F. DeLuca, T. Suda, and R. J. Cousins. 1971. Isolation and identification of 1,25-dihydroxycholecalciferol. A metabolite of vitamin D active in intestine. *Biochemistry*. **10**: 2799.
5. Haussler, M. R., D. W. Boyce, E. J. Littledike, and H. Rasmussen. 1971. A rapidly acting metabolite of vitamin D_3. *Proc. Natl. Acad. Sci. U. S. A.* **68**: 177.
6. Boyle, I. T., R. W. Gray, and H. F. DeLuca. 1971. Regulation by calcium of *in vivo* synthesis of 1,25-dihydroxycholecalciferol and 21,25-dihydroxycholecalciferol. *Proc. Natl. Acad. Sci. U. S. A.* **68**: 2131.
7. Shain, S. 1972. *In vitro* metabolism of 25-hydroxycholecalciferol by chick intestinal and renal cell preparations: identification of a metabolic product as 1,25-dihydroxycholecalciferol and delineation of its metabolic fate in intestinal cells. *J. Biol. Chem.* In press.
8. Potts, J. T., Jr., R. M. Buckle, L. M. Sherwood, C. F. Ramberg, Jr., C. P. Mayer, D. S. Kronfeld, L. J. Deftos, A. D. Care, and G. D. Aurbach. 1968. Control of secretion of parathyroid hormone. *In* Parathyroid Hormone and Thyrocalcitonin (Calcitonin). R. V. Talmage, and L. F. Belanger, editors. Excerpta Medica Foundation, New York. 407.
9. Nagata, N., and H. Rasmussen. 1970. Parathyroid hormone, 3'5' AMP, Ca^{2+}, and renal gluconeogenesis. *Proc. Natl. Acad. Sci. U. S. A.* **65**: 368.
10. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**: 265.
11. Hawker, C. D., J. D. Glass, and H. Rasmussen. 1966. Further studies on the isolation and characterization of parathyroid polypeptides. *Biochemistry*. **5**: 344.
12. Chase, L. R., and G. D. Aurbach. 1967. Parathyroid function and the renal excretion of 3',5'-adenylic acid. *Proc. Natl. Acad. Sci. U. S. A.* **58**: 518.
13. Birge, S. J., W. A. Peck, M. Berman, and G. D. Whedon. 1969. Study of calcium absorption in man: a kinetic analysis and physiological model. *J. Clin. Invest.* **48**: 1705.
14. Raisz, L. G., C. L. Trummel, M. F. Holick, and H. F. DeLuca. 1972. 1,25-dihydroxycholecalciferol: a potent stimulator of bone resorption in tissue culture. *Science (Wash. D. C.)*. **175**: 768.