Vol. 94, No. 1, 1980 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 14, 1980 Pages 313-318

LOCALIZATION OF 25-HYDROXYVITAMIN D_3-1α-HYDROXYLASE ACTIVITY IN THE MAMMALIAN KIDNEY

TAKASHI AKIBA*, HITOSHI ENDOU, CHIZUKO KOSEKI, and FUMINORI SAKAI**

Department of Pharmacology, Faculty of Medicine, University of Tokyo
7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan.

NOBORU HORIUCHI AND TATSUO SUDA

Department of Biochemistry, Showa University Dental School
1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142, Japan.

Received April 1,1980

Summary: Intra-renal distribution of 25-hydroxyvitamin D_3(25-OH-D_3)-1α-hydroxylase activity was studied in single nephron segments prepared from New Zealand White rabbit fetuses (26th to 28th day of gestation). Fetal kidneys were treated with collagenase and the isolated nephrons were micro-dissected into five different parts. Each segment of the nephron was incubated with [^{3}H]-25-OH-D_3. Metabolites of [^{3}H]-25-OH-D_3 were separated by high performance liquid chromatography. The results show that 25-OH-D_3-1α-hydroxylase activity is localized only in the proximal tubule and that the pars recta of proximal tubule possesses higher activity than the proximal convoluted tubule. The findings indicate that the major and probably exclusive site of 1α,25-$(OH)_2$-D_3 synthesis is the proximal tubule not only in birds, as reported by Brunette et al. (8), but also in mammals.

Introduction

It is now well established that the physiological functions of vitamin D are exerted by hydroxylated metabolites rather than the parent vitamin itself (1-2). Among several hydroxylated metabolites, 1α,25-dihydroxyvitamin D_3[1α,25-$(OH)_2$-D_3] is the most potent known metabolite in stimulating intestinal calcium transport and bone mineral mobilization(3,4). 1α,25-$(OH)_2$-D_3 has been thought to be synthesized exclusively in the kidney (5), though recent reports (6,7) demonstrate that placental tissues are also capable of producing 1α,25-$(OH)_2$-D_3

* Present address: Department of Internal Medicine, Tokyo Medical and Dental University, 1-5-45, Yushima, Bunkyo-ku, Tokyo 113, Japan.

** To whom all correspondence should be addressed.

Abbreviations used: 25-OH-D_3, 25-hydroxyvitamin D_3; 1α,25-$(OH)_2$-D_3, 1α,25-dihydroxyvitamin D_3; 24,25-$(OH)_2$-D_3, 24,25-dihydroxyvitamin D_3; PTH, parathyroid hormone; cyclic-AMP, adenosine 3'5'-monophosphate; Glm, glomerulus; PCT, proximal convoluted tubule; PR, pars recta of the proximal tubule; DT, distal tubule and thick ascending limb of Henle's loop; CT, collecting tubule.

0006-291X/80/090313-06$01.00/0

in pregnancy. Using single nephron preparations from rachitic chicks Brunette et al. (8) recently demonstrated that the major and the probably exclusive site of 1α,25-$(OH)_2$-D_3 synthesis is the proximal tubule. The precise intra-renal localization of 25-hydroxyvitamin D_3(25-OH-D_3)-1α-hydroxylase in mammals, however, is not known, because of the presence of the 1α-hydroxylase inhibitor in rats (9).

Very recently, Sunaga et al. (10) demonstrated that fetal kidneys from rabbits fed a laboratory chow can produce in vitro 1α,25-$(OH)_2$-D_3 from its precursor. Using single nephron preparations from the fetal rabbit kidney, we now report that 25-OH-D_3-1α-hydroxylase is localized primarily in the proximal tubule not only in birds, but also in mammals.

Material and Methods

Animals: Pregnant rabbits (New Zealand White strain) were obtained from local distributor. They were fed a rabbit chow (Oriental Co. Ltd., Tokyo) containing 0.85% calcium, 0.66% phosphorus and 1.0 U vitamin D_3/g diet ad libitum throughout pregnancy. On the 26th - 28th day of gestation, the animals were anesthetized with sodium pentobarbital (40 mg/kg), and the kidneys of the fetuses were perfused via the aorta with a collagenase solution which consisted of 0.1% collagenase (Sigma, Type 1), 1.0 mM $CaCl_2$ and 0.1% bovine serum albumin in a modified Hanks' medium (NaCl 137 mM, KCl 5 mM, $MgSO_4$ 0.8 mM, NaH_2PO_4 0.33 mM, KH_2PO_4 0.44 mM, $MgCl_2$ 1 mM, Tris-HCl 10 mM, pH 7.4).

Preparation of nephron segments: Slices cut along the corticopapillary axis were incubated in the same collagenase solution at 37°C for 50 min bubbled with 100% oxygen. The slices were rinsed 3 times with the ice cold modified Hanks' solution. Micro-dissection was performed in a petri-dish with a fine needle at 4°C under a stereomicroscope. Isolated nephrons were separated into 5 different portions. Proximal convoluted tubules (PCT) were collected from the proximal tubules attached to the glomerulus (Glm). The pars recta (PR) of the proximal tubules was micro-dissected from the inner cortex and outer medulla retrogradely from the early portion of its discending limb of Henle's loop. The thick ascending limb of Henle's loop and the distal tubule were micro-dissected from the attachment to the glomerulus at the macula densa. They were pooled and analysed togather as distal tubules (DT). Collecting tubules (CT) were micro-dissected from the cortex and outer medulla. Each nephron segment was identified by its location and appearance under a phase-contrast microscope. After each tubular segment was photographed for measurements of the length, they were transfered to a Terasaki's tissue calture plate with controlled humidity. The protein content of tubules was determined by Lowry's method (9).

Incubation: Each tubular segment was resuspended in 2 μl of an incubation medium. The medium was a modified Hanks' medium containing 5 mM pyruvate, 10 mM glucose and 140 nM [23,24-^{3}H]-25-OH-D_3(110 Ci/mmol, Radiochemical Centre, Amersham). Incubation was carried out at 37°C for 30 min and terminated by adding 10 μl of methanol. Extraction was performed according to the method of Bligh and Dyer (12).

Measurements of 25-OH-D_3-1α-hydroxylase activity: Chromatography of the extract was carried out on a high performance liquid chromatograph(Waters HPLC, Model 204 equipped with a Zorbax-SIL column 4.6 mm x 15 cm). The solvent system was either 1.5 % methanol in dichloromethane or 9% isopropanol in n-hexane. Fifty 30 sec fractions were collected into minivials. The radioactivity was determined with a Packard liquid scintillation counter (Model 3255). The metabolites of [^{3}H]-25-OH-D_3 were identified by co-chromatography with authentic 25-OH-D_3 or 1α,25-$(OH)_2$-D_3 on HPLC. Crystalline 1α,25-$(OH)_2$-D_3 was kindly donated by Dr. M.R. Uskokoić, Hoffman-LaRoche Inc., New Jersy. The amount of 1α,25-$(OH)_2$-D_3 produced was expressed in fmol/μg protein or fmol/mm of tubular length, based on the original specific activity of the [^{3}H]-25-OH-D_3.

Results

Figure 1 illustrates an HPLC profile of extracts of the PCT prepared from a rabbit fetus (on 28th day of gestation). The PCT metabolized [^{3}H]-25-OH-D_3 mainly to a polar peak, as reported by Sunaga et al. (8). The polar peak comigrated exactly the same position as authenthic 1α,25-$(OH)_2$-D_3.

The levels of 25-OH-D_3-1α-hydroxylase activity along nephron segments are shown in Table 1 and Figure 2. The only significant production of 1α,25-$(OH)_2$-D_3 occurred in the PCT and PR in each separate set of experiments (Table 1). Very little 1α,25-$(OH)_2$-D_3 was produced in the remaining parts of the

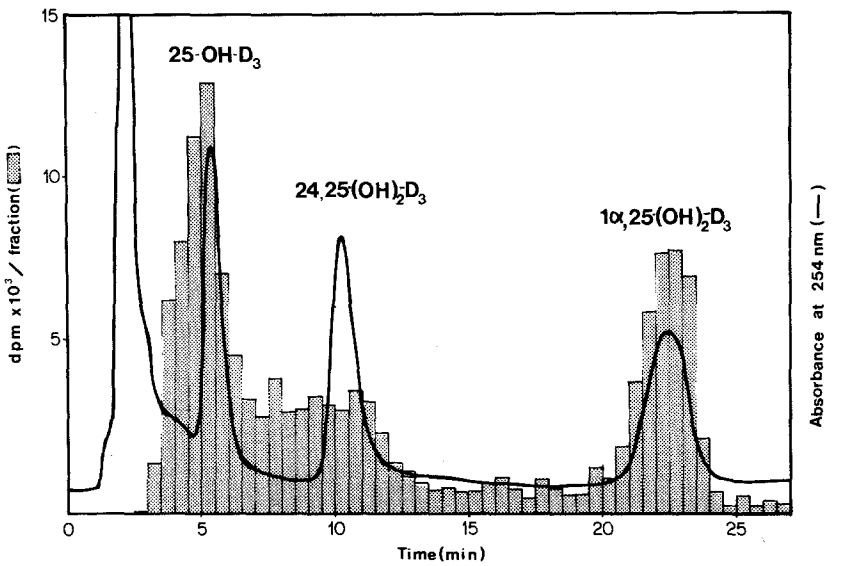

Fig. 1. HPLC profile of the extract of the PCT. Before applying to HPLC columns, 500 pmol of authentic 25-OH-D_3, 24,25-$(OH)_2$-D_3 and 1α,25-$(OH)_2$-D_3 were added to the sample. The columns were eluted with a solvent of 1.5% methanol in dichloromethane. The solid line indicates absorbance at 254 nm and the dotted bars represent radioactivity in each 30 sec fraction.

Table 1. 1α,25-$(OH)_2$-D_3 synthesis along the rabbit fetal nephron.

Experiments \ Nephron segments	Glm	PCT	PR	DT	CT
1	0.20	0.31	0.89	0	0
2	0	0.48	4.07	0	0
3	0	0.41	0.98	0	0
4	0.05	2.78	6.37	0.32	0
5	0	0.71	2.32	0	0
6	0	0.45	0.52	0	0

Values are expressed in fmol/glomerulus or fmol/mm tubular length.

nephron. Some 1α,25-$(OH)_2$-D_3 was synthesized by the Glm in Experiments 1 and 4. However, they were considered to be produced by contamitants of the PCT in the Glm. The amounts of 1α,25-$(OH)_2$-D_3 produced varied with each experiment, but the PR appeared to show higher activity than the PCT (Table 1). The mean amount of 1α,25-$(OH)_2$-D_3 synthesized was 3.67 ± 1.83 fmol/μg protein (o.85 ± 0.42

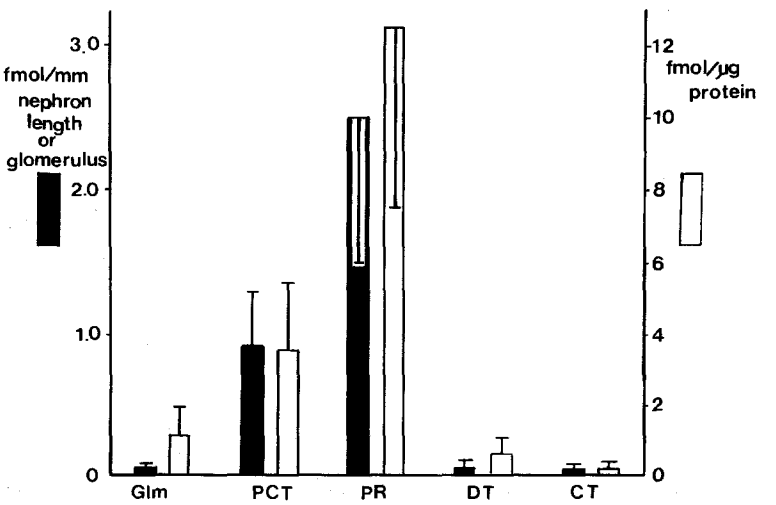

Fig. 2. 1α,25-$(OH)_2$-D_3 synthesis by various nephron segments prepared from fetal rabbit kidney. Columns indicate the mean value and the bars the standard errors of 6 separate sets of experiments. A significant amount of 1α,25-$(OH)_2$-D_3 was found only in the PCT and the PR. Results are expressed in fmol of 1α,25-$(OH)_2$-D_3 synthesized per mm of nephron length or fmol per glomerulus (■), and fmol per μg of tubular protein (□).

fmol/mm of nephron length, mean ± S.E. n=6) in the PCT and 12.5 ± 5.10 fmol/μg protein (2.52 ± 1.02 fmol/mm of tubular length) in the PR.

Discussion

The tubular preparations from rabbit fetuses made possible the first demonstration of the precise location of 25-OH-D_3-1α-hydroxylase activity in mammals. The result that the only significant production of 1α,25-$(OH)_2$-D_3 occurs in the proximal tubule is consistent with that of Brunette et al. (8), who demonstrated that 1α-hydroxylase activity was found only in the proximal convoluted tubules and thick loop cortex in rachitic birds. Their classification of chick nephron segments, however, appears to be different from that of the mammalian nephron.

In the rabbit fetal kidney, the PR showed higher 1α-hydroxylase activity than the PCT. These two portions of the rabbit nephron possess parathyroid hormone (PTH)-sensitive adenylate cyclase activity (13). According to the report of Chabardès et al.(13), The PR possesses higher adenylate cyclase activity induced by PTH than the PCT. PTH has been shown to be the most potent and the most important stimulator of 1α-hydroxylase activity. Cyclic AMP functions as an intracellular mediator in the PTH-induced stimulation of 1α-hydroxylase activity (14). Therefore, the higher 1α-hydroxylase activity in the PR than the PCT maybe explained by the difference in the PTH-sensitive adenylate cyclase activity.

The proximal tubule is a main site of inorganic phosphate reabsorption, which is also regulated by the PTH-cyclic AMP system (15). Renal 1α-hydroxylation of 25-OH-D_3 is induced by low plasma phosphate concentration (16,17) and, conversely, vitamin D or its metabolites increase renal reabsorption of inorganic phosphate (18,19). Therefore, it is interesting to consider the intracellular interactions of the two PTH-cyclic AMP sensitive functions, phosphate reabsorption and 1α,25-$(OH)_2$-D_3 synthesis.

The distribution of 25-OH-D_3-24-hydroxylase activity in nephron segments is of considerable interest in view of the control mechanism of 25-OH-D_3 metabolism (20), which is currently under investigation in our labolatories.

References

1. Omdahl, J.L., and DeLuca, H.F. (1973) Physiological Rev. 53, 327-372.
2. Norman, A.W., and Henly, H. (1974) Recent Progr. Hormone Res. 30, 431-480.
3. Boyle, I.T., Miravet, J., Gray, R.W., Holick, M.F., and DeLuca, H.F. (1972) Endocrinology 90, 605-608.
4. Raisz, L.G., Trummel, L., Holick, M.F. and DeLuca, H.F. (1972) Science (Washington) 175, 768-769.
5. Gray, R., Boyle, I.T., and DeLuca, H.F. (1971) Science (Washington) 172, 1232-1234.
6. Weisman, Y., Harell, A., Edelstein, S., David, M., Spirerer, Z., and Golander, A. (1979) Nature (London) 281, 317-319.
7. Tanaka,Y., Halloran, B., Shones, H.K., and DeLuca, H.F. (1979) Proc. Natl. Acad. Sci. USA 76, 5033-5035.
8. Brunette, M.G., Chan, M., Ferriere, C., and Roberts, K.D. (1978) Nature (London) 276, 287-289.
9. Botham, K.M., Tanaka,Y., and DeLuca, H.F. (1974) Biochemistry 13, 4961-4966.
10. Sunaga, S., Horiuchi, N., Takasaki, N., Okuyama, K., and Suda, T. (1979) Biochem. Biophys. Res. Commun. 90, 948-955.
11. Lowry, O.H., Rosenbrough, N.J., Farr, A.L., and Randall, R.F. (1951) J.Biol. Chem. 193, 265-275.
12. Bligh, E.G., and Dyer, W.J. (1959) Can. J. Biochem. Physiol. 37, 911-917.
13. Chabardès, D., Imbert, M., Clique, A., Montégut, M., and Morel, F. (1975) Pfügers Arch. 354, 229-239.
14. Horiuchi, N., Suda, T., Takahashi, H., Shimazawa, E., and Ogata, E. (1977) Endocrinology 101, 969-974.
15. Goldberg, M., Agus, Z.S., and Goldfarb, S. (1976) The Kidney pp.344-390, W.B. Saunders, Philadelphia.
16. Tanaka, Y., and DeLuca, H.F. (1973) Arch. Biochem. Biophys. 154, 556-574.
17. Baxter, L.A., and DeLuca, H.F. (1976) J. Biol. Chem. 251, 3158-3161.
18. Puschett, J.B., Fernandes, P.C., Boyle, I.T., Omdahl, J.L., and DeLuca, H.F. (1972) Proc. Soc. exp. Biol. Med. 141, 379-384.
19. Popovtzer, M.M., Robinette, J.B., DeLuca, H.F., and Holick, M.F. (1974) J. Clin. Invest. 53, 913-921.
20. Ghazarian, J.G. (1979) Vitamin D - Basic research and its clinical application. pp.587-596, Walter de Gruyter, New York.