Vol. 93, No. 1, 1980 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
March 13, 1980 Pages 149-154

25-OHD$_3$-26,23 LACTONE: DEMONSTRATION OF KIDNEY-DEPENDENT SYNTHESIS IN THE PIG AND RAT

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Received January 26,1980

SUMMARY

Plasma concentrations of polar metabolites of vitamin D$_3$ were measured in intact and bilaterally nephrectomized pigs and rats receiving large doses of vitamin D$_3$ and undergoing peritoneal dialysis. Plasma concentrations of 25-OHD$_3$ were increased one day after the vitamin D$_3$ injections. Bilaterally nephrectomized pigs had higher plasma concentrations of 25-OHD$_3$ and 25,26-(OH)$_2$D$_3$ throughout the experiment than control pigs had. Bilaterally nephrectomized rats, however, had lower concentrations of 25-OHD$_3$ and 25,26-(OH)$_2$D$_3$ than the control rats had. Both bilaterally nephrectomized pigs and rats were unable to produce 25-OHD$_3$-26,23 lactone (lactone) after a challenge of massive I.M. injections of vitamin D$_3$. Intact animals of both species had elevated concentrations of lactone (30-50 ng/ml) at the end of the experiment. Our results show that lactone, like 1,25-(OH)$_2$D$_3$, requires the kidney for its production.

INTRODUCTION

The metabolism of vitamin D$_3$ to more polar-active metabolites has been well established (1). Recently, a new metabolite of vitamin D$_3$ was observed in chick plasma during the course of preparing plasma lipid extracts for measuring 24,25-(OH)$_2$D$_3$ and 25,26-(OH)$_2$D$_3$ (2). This metabolite was referred to as peak X (2,3) and later identified as 25-OHD$_3$-26,23 lactone (4). Investigators at our Center have recently shown a method for purification and quantitation of this metabolite, and have documented its appearance in vitamin D$_3$-toxic pig and cow plasma (5). We now extend our observations to show that the kidney needs to be present for the synthesis of lactone in pigs and rats.

0006-291X/80/050149-06$01.00/0

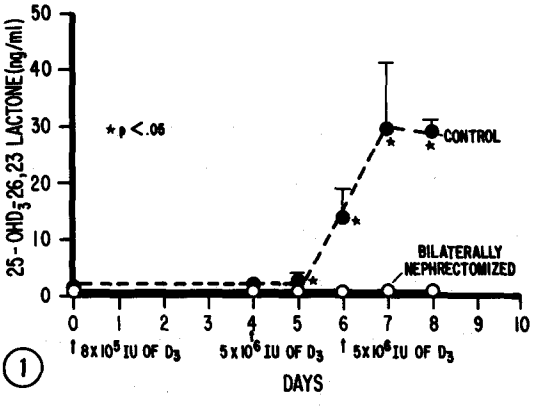
MATERIALS AND METHODS

Pigs -- Ten purebred Hampshire pigs were suckled for 3 weeks with no exposure to sunlight and constant access to creep feed after one week of age. After weaning (~ 3 weeks of age), they were fed a commercial pig development ration that supplied adequate calcium, phosphorus, and vitamin D_3 (6). At 6 weeks of age, one-half of the pigs were bilaterally nephrectomized and the other one-half were sham-operated under halothane anesthesia. Both groups had an equal number of males and females. All the pigs had a permanently implanted tube placed in the peritoneal cavity to facilitate peritoneal dialysis. Immediately after surgery (day 0), all the pigs in each group received 8 x 10^5 IU of vitamin D_3 in ethanol I.M. and 5 x 10^6 IU I.M. on days 4 and 6.

Rats -- Eight male albino rats (retired breeders from Holtzman, Madison, WI) were housed in hanging wire cages and received a vitamin D deficient diet (7). Under ether anesthesia, 4 of the rats were bilaterally nephrectomized and the other 4 sham-operated. Immediately after surgery, each rat received 8 x 10^5 IU of vitamin D_3 I.M. in ethanol.

All the animals (both bilaterally nephrectomized and controls) from each species were subjected to peritoneal dialysis once or twice daily with Ringer's solution containing 1.5-5.0% dextrose.

Blood samples -- Blood samples (10 ml from each pig and 1 ml from each rat) were taken at the intervals indicated in Figs. 1 and 2. Plasma was separated from red cells by centrifugation and stored frozen (-15°C) until analyzed. The plasma was analyzed by previously described methods for concentrations of 25-OHD_3, 25,26-$(OH)_2D_3$ (2), and lactone (5).



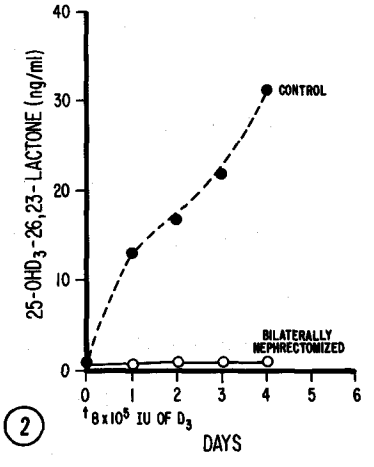


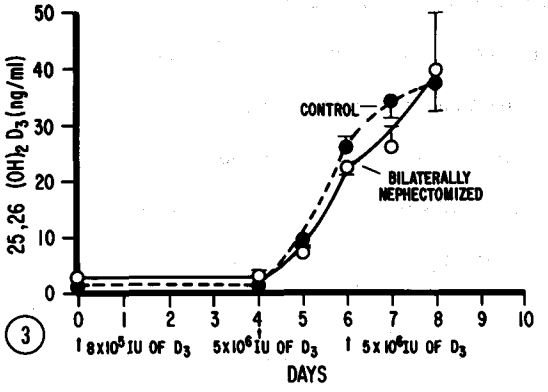
Figure 1. Plasma 25-OHD_3-26,23 lactone concentrations in intact (control) and bilaterally nephrectomized pigs treated with vitamin D_3. The bars represent the SE of the mean. The figure represents the levels in pigs treated with injections of 8 x 10^5 IU of vitamin D_3 on the day of surgery (day 0) followed by injections of 5 x 10^6 IU of vitamin D_3 on days 4 and 6.

Figure 2. Plasma 25-OHD_3-26,23 lactone concentrations in control and bilaterally nephrectomized rats treated with a single injection of 8 x 10^5 IU of vitamin D_3 on day 0.

RESULTS AND DISCUSSION

Intramuscular injections of vitamin D_3 failed to result in an elevation in plasma lactone in bilaterally nephrectomized pigs and rats undergoing peritoneal dialysis. The control animals, also subjected to dialysis, however, had elevated concentrations of this metabolite within 1-3 days after I.M. injections of vitamin D_3 (Figs. 1 and 2). Thus, the kidney appears to play an essential role in lactone production. Although this role has not been clearly defined, there are at least 3 possibilities: (1) kidney synthesis of precursors and the lactone produced elsewhere; (2) kidney synthesis of lactone and the precursor produced elsewhere; or (3) synthesis of both lactone and its precursor in the kidney.

The logical biochemical precursor to lactone is 25,26-$(OH)_2D_3$ because it would presumably require the fewest metabolic steps to be converted to the lactone. In our experiment, both the control and bilaterally nephrectomized pigs and rats had significant elevations in 25,26-$(OH)_2D_3$ concentrations after the vitamin D_3 injections (Figs. 3 and 4). These elevations confirm earlier results (3) that 25,26-$(OH)_2D_3$ can be produced extrarenally. By day 4, the intact rats had plasma concentrations of 25,26-$(OH)_2D_3$



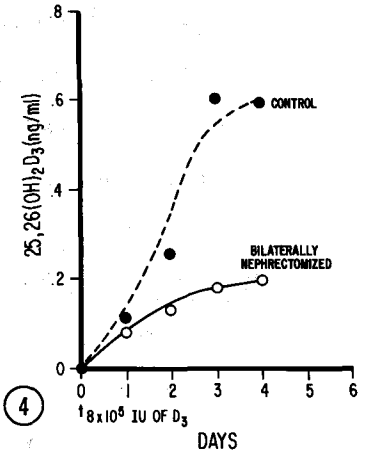
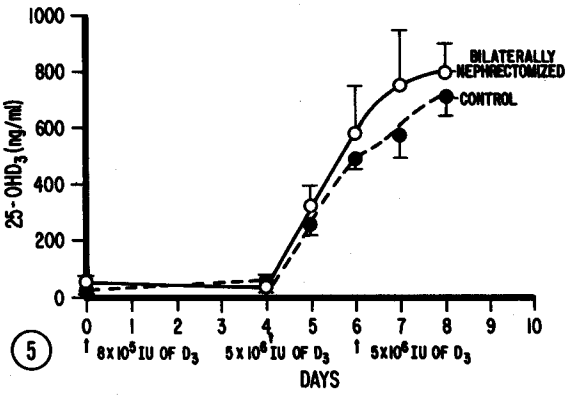


Figure 3. Plasma 25,26-$(OH)_2D_3$ concentrations in control and bilaterally nephrectomized pigs treated as shown in Fig. 1.

Figure 4. Plasma 25,26-$(OH)_2D_3$ concentrations in control and bilaterally nephrectomized rats treated as shown in Fig. 2.



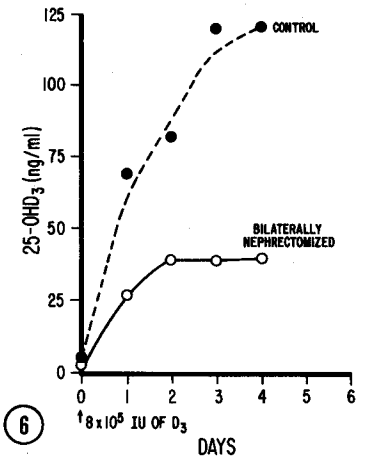


Figure 5. Plasma 25-OHD3 concentrations in control and bilaterally nephrectomized pigs treated as shown in Fig. 1.

Figure 6. Plasma 25-OHD3 concentrations in control and bilaterally nephrectomized rats treated as shown in Fig. 2.

(0.6 ng/ml) that were only 1/50 of its proposed metabolic product; i.e., lactone (30 ng/ml). These low concentrations of 25,26-$(OH)_2D_3$ would indicate that its turnover and conversion to the lactone is rapid or that another metabolite [possibly 23,25-$(OH)_2D_3$] acts as the major lactone precursor.

The 25-OHD_3 concentrations in the plasma are shown in Figs. 5 (pigs) and 6 (rats). The 25-OHD_3 concentration was higher in plasma from bilaterally nephrectomized pigs than in plasma from control pigs at all time points after vitamin D_3 administration (Fig. 5). Conversely, the 25-OHD_3 concentration was lower in plasma from nephrectomized rats than in plasma from intact rats (Fig. 6). The reason for this difference is unknown. A possible reason could be that the rats were anesthetized daily with ether before bleeding and dialysis. This daily exposure to ether may have severely depressed the ability of nephrectomized rats to hydroxylate vitamin D_3.

Although the biological activity of lactone has not been determined, a few speculative points can be made as to its biological significance. Results at our Center have shown that this metabolite is preferentially bound 5:1 by the binding sites on the rat plasma vitamin D_3 binding protein when com-

pared with other major circulating vitamin D_3 metabolites [25-OHD_3, 24,25-$(OH)_2D_3$ and 25,26-$(OH)_2D_3$] (5). Therefore, as the plasma concentrations of lactone increase, the ability of other metabolites to bind to the carrier protein may be compromised. This effect may be minimal because only 5-7% of the binding capacity of the carrier protein is used (8) under normal conditions. However, by competing more efficiently for plasma binding sites, an elevation in lactone such as during vitamin D_3 toxicity (5) may have the net result of increasing the amount of free or weakly bound 25-OHD and other vitamin D metabolites known to simulate 1,25-$(OH)_2D$ activity (9,10). This increase in free metabolites could promote an increased degradation and thus a reduced toxicity. Conversely, an increase in free metabolites could increase their availability to cellular receptors and thus promote toxicity. The predominant influence of lactone on these processes is currently being investigated at our Center.

CONCLUSION

Our results in the pig and rat, along with our previous observations (5) of [^{3}H]-lactone production in vitro in chick kidney homogenates, would indicate that lactone and its precursors are produced in the kidney. However, if significant lactone can be produced extrarenally, the lack of production in nephrectomized animals could be due to lack of the kidney-produced precursor.

ACKNOWLEDGMENTS

The authors would like to thank C. A. Hauber, R. L. Lyon, R. D. Evans, and P. A. Herrig for their excellent technical assistance.

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