Comparison of Activity of 25-Hydroxycholecalciferol and Dihydrotachysterol$_2$ in the Thyroparathyroidectomized Rat[1] (35276)

HELEN C. HARRISON AND HAROLD E. HARRISON

Departments of Pediatrics, Johns Hopkins University School of Medicine and Baltimore City Hospitals, Baltimore, Maryland 21205

It is possible to consider the actions of vitamin D in terms of physiologic and pharmacologic effects. In man, 2.5 to 10 μg/day suffice to meet physiologic requirements; whereas amounts in excess of 1 mg/day are needed to replace parathyroid hormone in the treatment of hypoparathyroidism. Comparison of the action of vitamin D_2, ergocalciferol, with that of dihydrotachysterol$_2$, another product of irradiation of ergosterol, suggests that the mechanism of action of vitamin D in physiologic doses differs from the mode of action in pharmacologic doses. In the hypoparathyroid rat, dihydrotachysterol$_2$ is much more effective in raising the serum calcium concentration than is ergocalciferol (1) although it is much less potent than ergocalciferol in raising the concentration of calcium in the serum of rats hypocalcemic because of vitamin D deficiency (1). In the former instance, the activated sterols are functioning as a substitute for parathyroid hormone and the action is therefore a pharmacologic one, requiring amounts of vitamin D several hundredfold greater than the physiologic requirement. In the latter case the activated sterols are acting with endogenous parathyroid hormone to permit the full expression of the action of this hormone on target tissues and this is one of the physiologic roles of vitamin D (2). Blunt *et al.* (3) have shown that Vitamin D undergoes a metabolic transformation before exerting its normal physiologic action and the initial metabolic transformation product is 25-hydroxycalciferol. It is not known, however, whether 25-hydroxylation is a necessary step in the pharmacologic action of vitamin D. If this were the case, and if 25-hydroxylation were a rate-limiting reaction in the conversion of calciferol to an active compound, the 25-hydroxy compound should be more potent than calciferol in elevating the serum calcium of the hypoparathyroid rat. Synthetic 25-hydroxycholecalciferol was therefore assayed for this effect.

Methods. The experiments were performed as previously described (1). Male rats 6 to 7 weeks of age weighing approximately 120 g were thyroparathyroidectomized under ether anesthesia. The animals had been maintained from weaning on a diet containing 0.5% calcium with a calcium: phosphorus ratio of 1.25. They had been given a total of 1000 units of vitamin D_2 in divided doses during the 3 weeks postweaning. They were continued on the same diet postoperatively and were given, in addition as drinking water, a solution of 1% calcium gluconate in 2.5% glucose for the first 4 or 5 days following thyroparathyroidectomy. This solution was replaced by 0.45% sodium chloride in 2.5% glucose and 24 hr later the rats were bled from the tail vein and determinations of calcium were made on the serum. Rats with serum calcium concentrations below 7.5 mg/ml were classified as parathyroidectomized; whereas those with serum calcium concentrations above 9 mg/100 ml were considered to have residual functioning parathyroid tissue and were used as controls. The parathyroidectomized rats were divided into groups and given either 25-hydroxycholecalciferol[2] or dihydrotachysterol$_2$ dissolved in propylene glycol by stomach tube in the

[1] This work was supported by National Institutes of Health Grant AM-00668.

[2] 25-Hydroxycholecalciferol was supplied to us by Dr. John Babcock, the Upjohn Company.

TABLE I. Response of Thyroparathyroidectomized (TXPTX) Rats to 25-Hydroxycholecalciferol and to Crystalline Dihydrotachysterol$_2$.

Group (μg)	Serum (mg/100 ml)			
	Pretreatment	Posttreatment		
	Ca	Ca	P	Mg
Operated controls	10.4 ± 0.13[a]	10.1 ± 0.12	7.6 ± 0.20	1.53 ± 0.04
TXPTX	6.7 ± 0.36	6.3 ± 0.26	12.2 ± 0.50	0.92 ± 0.04
25-OHCC				
100	6.7 ± 0.28	7.9 ± 0.20	11.4 ± 0.20	1.00 ± 0.06
250	7.1 ± 0.23	7.9 ± 0.28	9.7 ± 0.18	1.00 ± 0.06
500	6.9 ± 0.23	9.8 ± 0.30	9.6 ± 0.30	1.20 ± 0.05
DHT, 100[a]	7.1 ± 0.29	10.4 ± 0.36	10.3 ± 0.63	1.07 ± 0.03
100[b]		10.5 ± 0.83	11.0 ± 0.93	1.00 ± 0.30
Vit. D_2, 500[b]		9.9 ± 1.47	11.6 ± 0.46	0.88 ± 0.09

[a] Means ± standard error of mean.

[b] These data taken from previous report (1) are means ± 95% confidence limits.

doses listed in Table I. 48 hr later the animals were bled from the aorta under pentobarbital anesthesia and determinations of the concentration of calcium, phosphorus, and magnesium in the serum were made by methods previously described (1).

Table I lists the concentrations of calcium in the serum of animals in the various treatment groups both before treatment and 48 hr after the indicated sterol dose. The concentrations of phosphorus and magnesium in the serum posttreatment are also given. The controls, *i.e.*, operated animals with intact parathyroids, and the untreated thyroparathyroidectomized rats show no significant change in concentration of serum calcium as a result of the experimental procedure. The effect of parathyroid deficiency in increasing the serum phosphorus and reducing the serum magnesium concentrations is apparent. 100 μg of 25-hydroxycholecalciferol does produce a significant elevation of serum calcium concentration but a dose of 500 μg is required to bring the calcium concentration to the normal range. At this dose the serum phosphorus concentration is reduced and the serum magnesium concentration increased but not to the concentrations of control rats with normal parathyroid function. 100 μg of dihydrotachysterol$_2$ increases the serum calcium concentration to control values and is equivalent in this respect to 500 μg of 25-hydroxycholecalciferol. The potency ratio between dihydrotachysterol$_2$ and 25-hydroxycholecalciferol is probably less than 5 when measured by the effects on the concentrations of phosphate and magnesium in serum. Comparison of these results with 25-hydroxycholecalciferol with the previously reported data (1) of similar experiments with ergocalciferol, (vitamin D_2) and dihydrotachysterol$_2$ shows that there is no detectable difference between the effectiveness of 25-hydroxycholecalciferol and ergocalciferol in raising the serum calcium concentration of parathyroid-deficient rats and that both compounds are only about $\frac{1}{5}$ as potent as dihydrotachysterol$_2$ in this assay.

Table II shows the effect of 25-hydroxycholecalciferol on the serum calcium concentration of hypocalcemic vitamin D depleted rats as well as on *in vitro* transport of calcium by everted duodenal sacs as measured by the concentration ratio of 45 calcium in serosal to mucosal fluid (C_s/C_m) following incubation. The treated rats were given 0.1 μg of 25-hydroxycholecalciferol in propylene glycol by stomach tube and the untreated controls were given only the solvent. 72 hr later the rats were bled and duodenum was taken for everted loops as described before (4). 0.1 μg of 25-hydroxycholecalciferol

TABLE II. Effect of 25-Hydroxycholecalciferol in Hypocalcemic Intact Rats.[a]

Group	Serum (mg/100 ml)				Duodenal transport C_s/C_m
	Pretreatment	Posttreatment			
	Ca	Ca	P	Mg	
25-OHCC, 0.1 μg	5.6	7.5	8.9	1.37	2.15
	[5.4–5.8]	[6.5–8.2]	[8.1–10.1]	[1.30–1.42]	[1.93–2.38]
	(4)	(4)	(4)	(4)	(4)
Controls	5.2	5.2	9.0	1.21	1.23
	[4.5–5.7]	[4.9–5.4]	[8.7–9.2]	[1.07–1.47]	[1.01–1.36]
	(4)	(4)	(4)	(4)	(4)

[a] Values are means with range in brackets; no. of animals in each group is given in parentheses.

effects a significant increase in serum calcium concentration in comparison with controls and also increases the *in vitro* duodenal transport of calcium. These effects are essentially the same as those produced by 0.1 μg of ergocalciferol in previous experiments (1).

In these experiments 25-hydroxycholecalciferol shows a physiologic effect in vitamin D-depleted rats in a dose of 0.1 μg. We did not assay the 25-hydroxycholecalciferol preparation for vitamin D potency by standard vitamin D assays. Such studies have been made by Blunt *et al.* (5), who found that 25-hydroxycholecalciferol had a potency of 56 IU/μg; whereas that of ergocalciferol is 40 IU/μg . The pharmacologic potency of 25-hydroxycholecalciferol assayed in the thyroparathyroidectomized rats was approximately the same as ergocalciferol and both compounds had about ⅕ the activity of dihydrotachysterol$_2$. These results do not indicate that the lesser effectiveness of ergocalciferol in comparison with dihydrotachysterol$_2$ in the hypoparathyroid rat is due to a limitation in the rate of 25-hydroxlation. Recent studies by Suda and colleagues (6) have indicated that 25-hydroxydihydrotachysterol$_3$ is about twice as potent as dihydrotachysterol$_3$ as measured by the serum calcium-raising effect in the thyroparathyroidectomized rat. They suggest that dihydrotachysterol also undergoes 25-hydroxylation and that this could be essential for its physiologic activity. The greater potency of dihydrotachysterol in comparison with either ergocalciferol or 25-hydroxycholecalciferol in the absence of parathyroid hormone suggests that the pharmacologic action of vitamin D and related sterols is exerted through a mechanism different from the physiologic effect of these compounds. The physiologic action is believed to result from the activation, by vitamin D, of DNA-directed mRNA synthesis and induction of a protein or proteins which function in the transcellular transport of calcium (7). The nature of the pharmacologic action is unknown. Parathyroid hormone activates an adenyl cyclase in renal cortex (8) and bone (9) increasing the formation of 3′,5′-cyclic adenosine monophosphate (cyclic AMP) which has been postulated to be the mediator of parathyroid hormone action in the target cells. The effect of pharmacologic doses of activated sterols upon cyclic AMP production by target issues of the hypoparathyroid animal has not been determined.

Summary. Assays of the activty of synthetic 25-hydroxycholecalciferol in the thyroparathyroidectomized rat indicate that it is no more potent than ergocalciferol. These and other data (1) indicate that both 25-hydroxycholecalciferol and erygocalciferol are only ⅕ as active as dihydrotachysterol$_2$ in this assay. These results suggest that the pharmacologic action of vitamin D as a substitute for parathyroid hormone operates through a mechanism different from the physiologic action of this sterol derivative.

1. Harrison, H. E., Harrison, H. C., and Lifshitz, F., *in* "Parathyroid Hormone and Thyrocalcitonin (Calcitonin)" (R. V. Talmage and L. F. Belanger,

eds.), p. 455. Excerpta Med. Found., New York (1968).

2. Harrison, H. E., and Harrison, H. C., Metabolism **13**, 952 (1964).

3. Blunt, J. W., DeLuca, H. F., and Schnoes, H. K., Biochemistry **7**, 3317 (1968).

4. Harrison, H. E., and Harrison, H. C., Amer. J. Physiol. **199**, 265 (1960).

5. Blunt, J. W., Tanaka, Y., and DeLuca, H. F., Proc. Nat. Acad. Sci. U.S.A. **61**, 1503 (1968).

6. Suda, T., Hallick, R. B., DeLuca, H. F., and Schnoes, H. K., Biochemistry **9**, 1651 (1970).

7. Norman, A. W., Biol. Rev. **43**, 97 (1968).

8. Chase, L. R., and Aurbach, C. D., Science **159**, 548 (1968).

9. Chase, L. R., Fedak, S. A., and Aurbach, C. D., Endocrinology **84**, 761 (1969).

Received Sept. 2, 1970. P.S.E.B.M., 1971, Vol. 136.