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Comparison of the Effects of Vitamin D_3, Dihydrotachysterol, and Parathormone on Calcium Kinetics in the Rat*

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Summary: Rats were treated with 100 I.U. (= 2.5 mcg) Vit. D_3/day during 14 days and submitted to a radio-Calcium assay of Calcium kinetics. The results were compared with those obtained in weight-matched pair-fed controls, and with those obtained in former experiments applying dihydrotachysterol and parathormone. Vit. D_3, which is transformed to a hormone, increased the fast exchange compartment, the exchange rate between the compartments, the endogenous fecal Calcium excretion rate, and the bone uptake and release rates. These findings indicate that the Vit. D-hormone is more similar in its action to the other hormone, parathormone, than to its close chemical relative dihydrotachysterol.

Key words: Calcium kinetics – vitamin D_3 – dihydrotachysterol – parathormone – rat

Zusammenfassung: Ratten wurden mit 100 I.E. (= 2,5 µg) Vit. D_3/Tag über 14 Tage behandelt und dann einer Untersuchung ihrer Calcium-Kinetik mit Radio-Calcium unterworfen. Die Ergebnisse wurden mit denen bei gleichgewichtigen paargefütterten Kontrollen und mit denen früherer Experimente mit Dihydrotachysterin und mit Parathormon verglichen. Vit. D_3, das in ein Hormon verwandelt wird, vergrößerte das schnell austauschende Compartiment, die Austauschrate zwischen den Compartimenten, die Ausscheidungsrate des endogenen fäkalen Calciums und die Knochen- Aufnahme- und -Abgabe-Raten. Diese Befunde zeigen, daß das Vit. D-Hormon dem anderen Hormon, Parathormon, ähnlicher im Verhalten ist als seinem nahen chemischen Verwandten Dihydrotachyterin.

Schlüsselwörter: Vitamin D – Dihydrotachysterin – Parathormon – Calcium-Kinetik – Ratte

In former work we have investigated the influence of dihydrotachysterol (DHT) (REMAGEN, 1970) and parathyroid hormone (PTH) (SCHULZ *et al.*, 1971) overdosages on the kinetics of Calcium (Ca) metabolism in the rat, applying the two-compartment model of AUBERT and MILHAUD (1960) and using 45Ca as tracer. Under DHT treatment we observed an increase of all parameters of Ca metabolism, with the exception of the bone turnover rates. PTH, to the contrary, increased only

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the pool size, and bone turnover in particular. In consideration of these differences between the two compounds, we examined the influence of elevated Vitamin D_3 (Vit. D) doses on the parameters of the model of rat Ca metabolism, and compared the results with those obtained with DHT and PTH.

MATERIAL AND METHODS

Female albino rats of the Füllinsdorf outbred stock[2] (130 g body weight) received daily doses of 100 I.U. (= 2.5 mcg) Vit. D_3 dissolved in sunflower oil by gavage during 14 days. On the last three days of treatment they were submitted to the radio-Ca assay of Ca kinetics designed by AUBERT and MILHAUD (1960), and by MILHAUD *et al.* (1960), which is described in detail in the preceeding paper (REMAGEN *et al.*, 1975). The radioactivity curve in the serum was established by measurements 2, 4, 6, 24, 32, and 48 hours after the intravenous injection of the tracer. Radioactivity measurements in sera and ash solutions were done by liquid scintillation counting, the stable Ca contents were determined by atomic absorption spectrophotometry (GUNCAGA and HAAS, 1973). The treated animals were compared with weight-matched pairfed controls. The values for the parameters of the model and the statistical evaluations with application of STUDENTs t-test, were calculated on the Sandoz Univac computer system using two programs coded in Fortran IV.

RESULTS

In Tab. 1., the mean values with standard deviations for the parameters of the Ca metabolism model are listed. Vit. D provoked a highly significant elevation of the serum Ca level. Whereas the increase in size of the fast exchange compartment was just on the borderline, the exchange between the compartments, and the total loss from the pool showed highly significant faster rates. The latter is due to an increase in endogenous fecal excretion and to a higher Ca deposition rate in bone. Ca mobilization from bone was also significantly increased over the normal value.

DISCUSSION

The Vit. D dose applied in our experiments is deduced from the observation of CARLSSON and LINDQUIST (1955) that 100 I.U. of Vit. D had the same effect on the serum Ca level of rats as 0.03 mg of DHT, which dose we had used in former experiments (REMAGEN, 1970). In our animals, which were fed a diet rich in Ca, we never reached the same serum Ca level with Vit. D as with DHT, in contrast to the rats of CARLSSON and LINDQUIST, which were habitually on a low Ca diet.

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[2] Kindly supplied by Fa. Hoffmann-La Roche, Ltd., Basle

Table 1. *Parameters of the model; mean values ± SD (6 animals in each group)*

Parameter		Vit.D		controls
Serum Calcium	S	11.23 ± 0.13	mg%	10.71*** ± 0.06
Body weight	G	135.00 ± 2.00	g	130.50 ± 1.63
Ca intake with food	v_i	94.26 ± 5.12	mg/d	88.29 ± 3.18
total fecal loss	v_F	82.63 ± 5.97	mg/d	70.38 ± 4.72
Urin. excret. chem. estim.	v_{uexp}	1.55 ± 0.18	mg/d	3.22 ± 0.83
Compartment fast exchange	P	24.87 ± 1.35	mg	21.61 ± 0.71
Compartment slow exchange	E	52.41 ± 2.56	mg	48.59 ± 3.69
Exchange P - E	V_e	93.05 ± 3.35	mg/d	75.98*** ± 2.99
total loss from P	v_T	86.79 ± 2.06	mg/d	75.18*** ± 2.08
Urinary excretion	v_u	0.66 ± 0.16	mg/d	1.03 ± 0.31
endogenous fecal Ca	v_f	10.79 ± 0.94	mg/d	6.37*** ± 0.61
bone accretion	v_{o+}	75.34 ± 1.95	mg/d	67.78* ± 2.26
bone resorption	v_{o-}	64.37 ± 1.72	mg/d	50.89* ± 5.09
intestinal absorption	v_a	22.42 ± 1.60	mg/d	24.29 ± 5.67
percent absorption	α	24.01 ± 1.80	%	26.94 ± 6.08
overall balance	Δ	+ 10.98 ± 2.13	mg/d	+ 16.89 ± 6.17

*= Difference between means of Vit.D treated and control animals statistically significant: * $p < 0.05$, ** $p < 0.025$, *** $p < 0.005$

In Fig. 1, the values observed unter Vit. D, DHT, and PTH treatment for the parameters are expressed in percent of their respective controls. In the two-compartment model used in all these investigations, serum Ca is a part of the fast exchange compartment (P), which was enlarged in two of the three conditions, DHT, and PTH overdosage. Only PTH also increased the compartment of

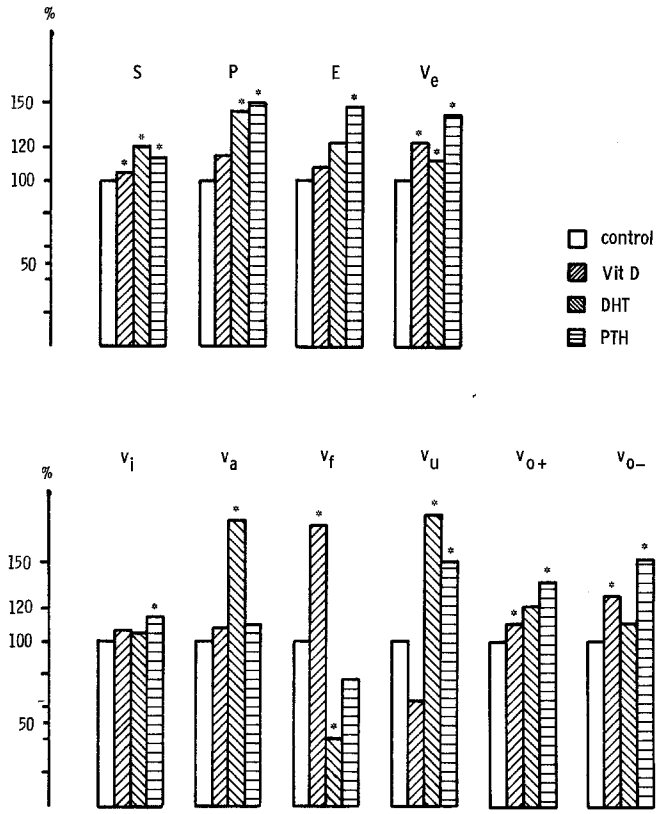

Fig. 1. Mean values for the parameters of the calcium metabolism model, expressed as percent of the control values set at 100%, and compared with DHT (REMAGEN, 1970), and with PTH (SCHULZ *et al.*, 1971) values presented in the same manner; * = mean value significantly different from corresponding control value

slow exchange (E). The exchange rate (V_e) between the compartments was higher in all the three experiments. Both the Vit. D and the DHT rats were pairfed with their controls, but the latter were on a diet with a reduced Ca content during the radio-Ca assay. It follows that comparing Ca intake (v_i), absorption (v_a), and its percentage, as well as Ca excretion with urine (v_u) and faeces (v_F) of the two groups would not be meaningful. The PTH rats for their part had a Ca intake (v_i) just significantly higher than their controls, but intestinal absorption (v_a) and its percentage, as well as the endogenous excretion (v_f) did not differ. Now it is interesting to note that in the DHT rats the endogenous excretion was significantly decreased, in comparison with their controls. This was explained to be due to an increased Ca reabsorption from the bowel. The Vit. D animals, to the contrary, excreted significantly more

Ca through their intestine than their controls. On the base of our present knowledge (DELUCA, 1972; BORLE, 1973) the different kind of activity of the two related compounds may be explained as follows: The Vit. D rats were on a diet rich in Ca and supplemented with Vit. D. Unter these circumstances, formation of 1,25-$(OH)_2$-Vit. D_3 is reduced, probably by the elevated Ca content of the mitochondria, were hydroxylation takes place. Thus, intestinal absorption is not sensibly altered by additional Vit. D. The higher endogenous intestinal excretion is difficult to explain under these circumstances; it may possibly be called forth by the elevated serum Ca level, but we admit that this explanation is not entirely satisfactory. DHT for its part is hydroxylated to its active form (25-DHT) only in the liver, and proportionally to the dose given. As there is no negative feedback control, and the hydroxylating system is different from that for Vit. D, the active form is produced in great quantities. This explains its greater effectiveness, compared with Vit. D in general, and in our experiments in particular, where it stimulated the reabsorption of the endogenous Ca.

Vit. D and PTH both influence bone turnover by increasing the accretion and resorption rates. DHT, to the contrary, only increases bone resorption, on the condition that intestinal absorption is insufficient and to the extent necessary to counterbalance that insufficiency. In the Vit. D rats, bone histology does not show any morphological equivalent to the increased bone turnover rates (v_{o+} and v_{o-}). AUBERT and MILHAUD (1960) felt that the bone turnover rates include slow exchange processes upt to 25%. Probably these exchange movements which are paralleled by the increased exchange rate between P and E (V_e), are responsible for the increase of the turnover rates. From our radiochemical data we cannot positively answer this question, but the lack of morphological changes could thus be explained. PTH overdosage, to the contrary, calls forth morphological alterations in the bone which may be correlated to the radiochemical results. Obviously, these morphological findings do not exclude exchange processes being included in the increased bone turnover rates, and paralleling the particularly high exchange rate between the compartments (V_e) in this condition.

The considerable differences between the isotope (v_u) und chemical (v_{uexp}) estimations of urine Ca are due to the very small total quantities, which may involve an error in measurement up to 50%. Notwithstanding these differences there is a statistically significant correlation between the values obtained by the two methods in the control group.

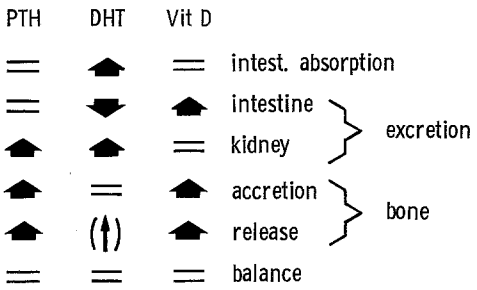

Fig. 2. Display of effects of the 3 compounds on the vectors of calcium metabolism; big arrows = statistically significant changes, horizontal lines = no significant changes, small arrow in brackets = changes conditioned by intestinal absorption

Our findings are sketched in Fig. 2: Vit. D, DHT, and PTH overdosages rise the serum Ca level by influencing the parameters of Ca metabolism in different ways; concerning intestinal Ca absorption, their actions differ as only DHT is operative, concerning bone turnover, the actions of Vit. D and PTH resemble one another, whereas DHT again acts in a different manner. We conclude that Vit. D which is transformed to a hormone, is more similar in its action to the other hormone, PTH, than to its close chemical relative DHT.

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