BIOLOGICAL ACTIVITY ASSESSMENT OF 25-HYDROXYVITAMIN D_3-26,23-LACTONE IN THE RAT

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1. Introduction

A new metabolite of vitamin D_3 has been isolated from the plasma of chickens, rats and pigs given large doses of vitamin D_3 and identified as 25-hydroxyvitamin D_3-26,23-lactone (25-OH-D_3-26,23-lactone) [1–3]. Four possible diastereoisomers of 25-OH-D_3-26,23-lactone have been synthesized [4–9]; they have been directly compared to the natural 25-OH-D_3-26,23-lactone by high pressure liquid chromatography. The stereochemical configurations of the natural 25-OH-D_3-26,23-lactone at C-23 and C-25 positions were determined to be 23(*S*) and 25(*R*), respectively [9,10]. 23(*S*)25-Dihydroxyvitamin D_3 [23(*S*)25-$(OH)_2D_3$] is a far better substrate for production of 25-OH-D_3-26,23-lactone than is 25,26-dihydroxyvitamin D_3 (25,26-$(OH)_2D_3$) [11]. 23(*S*)25(*R*)26-trihydroxyvitamin D_3 [23(*S*)25(*R*)26-$(OH)_3D_3$] is a better substrate than 23(*S*)25-$(OH)_2D_3$ in the biosynthesis of the 25-OH-D_3-26,23-lactone [12]. Therefore, the 25-OH-D_3-26,23-lactone may be biosynthesized from 25-OH-D_3 by way of 23(*S*)25-$(OH)_2D_3$ to 23(*S*)25-(*R*)26-$(OH)_3D_3$.

The biological activity and physiological function(s) of the 25-OH–D_3-26,23-lactone are still unknown. Here, we report that in the rat that 25-OH-D_3-26,23-lactone slightly stimulates intestinal calcium transport and at the same time mediates a fall in the concentration of serum calcium.

Abbreviations: 25-OH-D_3, 25-hydroxyvitamin D_3; 25-OH-D_3-26,23-lactone, 25-hydroxyvitamin D_3-26,23 lactone; 23,25-$(OH)_2D_3$, 23,25-dihydroxyvitamin D_3; 25,26-$(OH)_2D_3$, 25,26-dihydroxyvitamin D_3; 1α,25-$(OH)_2D_3$, 1α,25-dihydroxyvitamin D_3; 23,25,26-$(OH)_3D_3$, 23,25,26-trihydroxyvitamin D_3; 1α, 25-$(OH)_2D_3$-26,23-lactone, 1α,25-dihydroxyvitamin D_3-26,23-lactone

2. Materials and methods

2.1. *Compounds*

We synthesized 25-OH-D_3 and 1α,25-$(OH)_2D_3$ as in [13]. 25-OH-D_3-26,23-Lactone was isolated and purified from the serum of rats given large doses of vitamin D_3 as in [10].

2.2. *Assay for intestinal calcium transport and bone calcium mobilization*

Male weanling rats of the Wistar strain were fed a vitamin D-deficient low calcium diet (Ca, 0.0036%; P, 0.3%; Teklab Test Diet, Madison WI) for 6 weeks. At the end of week 6, 5 rats each (~100 g body wt) received an intravenous injection of 500 ng 25-OH-D_3-26,23-lactone, 250 ng 1α,25-$(OH)_2D_3$ or 500 ng 25-OH-D_3 in 0.2 ml 0.2% Triton X-100 solution. The rats were sacrificed at the indicated times after administration, and the intestinal calcium transport and serum calcium concentration were measured. The intestinal calcium transport assay using everted duodenal sacs was done as in [14]. Serum calcium concentration was determined by the OCPC (*o*-cresolphthalein complexone) method [15].

3. Results

The biological activity of the isolated 25-OH-D_3-26,23-lactone was assayed in vitamin D-deficient rats fed a low calcium diet. Initially, a single dose of 500 ng 25-OH-D_3-26,23-lactone was used to test its intestinal calcium transport activity in comparison to that of 1α,25-$(OH)_2D_3$ or 25-OH-D_3 (fig.1). The 25-OH-D_3-26,23-lactone slightly stimulated intestinal calcium transport after 12 h and it reached a maxi-

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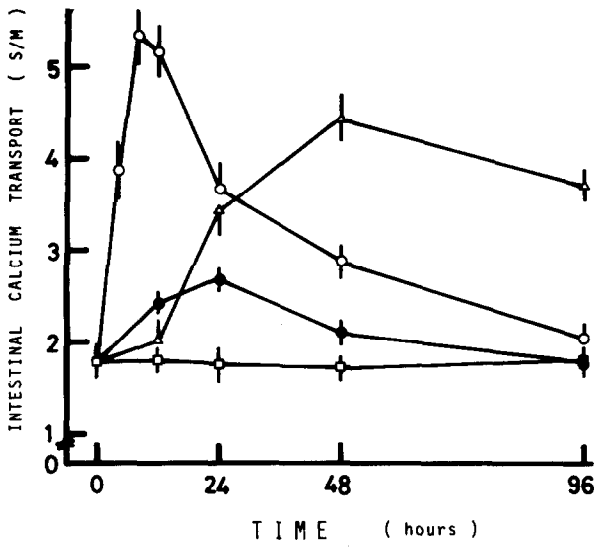

Fig.1. Time-course response of intestinal calcium-transport system. Rats on a low calcium diet received a single intravenous dose of either 500 ng 25-OH-D_3-26,23-lactone (•) or 250 ng 1α,25-$(OH)_2D_3$ (○) or 500 ng 25-OH-D_3 (△) or vehicle (□) in 0.2 ml 0.2% Triton X-100 solution. At the indicated times, animals were decapitated and their duodena were used for the determination of intestinal calcium transport. The rate of intestinal calcium transport is represented by the ratio of $^{45}Ca^{2+}$ in the serosal medium to $^{45}Ca^{2+}$ in the mucosal medium. Each point is the mean ± SEM of determinations on 5 rats.

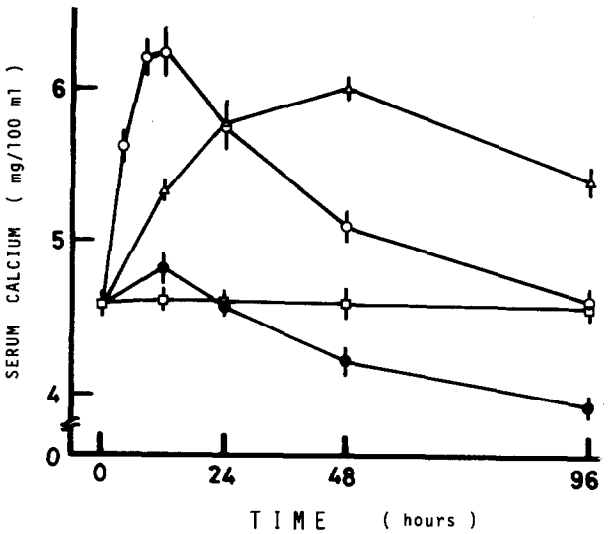

Fig.2. Time-course response of bone calcium mobilization induced in rats by 500 ng 25-OH-D_3-26,23-lactone (•) or 250 ng 1α,25-$(OH)_2D_3$ (○) or 500 ng 25-OH-D_3 (△) or vehicle (□). Rats on a low calcium diet received a single intravenous dose of compound in 0.2 ml 0.2% Triton X-100 solution. At the indicated times, animals were decapitated, blood was collected, and calcium was measured in the serum by the OCPC method [15]. Data are expressed as mg Ca^{2+}/100 ml serum and are the mean ± SEM of 5 determinations.

mum 24 h after administration. After 48 h, the responses did not differ significantly from the control. On the contrary, 1α,25-$(OH)_2D_3$ and 25-OH-D_3 demonstrated a greater stimulation of intestinal calcium transport than did 25-OH-D_3-26,23-lactone.

The time course of calcium mobilization from bone, as measured by an elevation in serum Ca^{2+} levels, produced by 500 ng 25-OH-D_3-26,23-lactone was compared to that produced by 250 ng 1α,25-$(OH)_2D_3$ or 500 ng 25-OH-D_3 (fig.2). The 25-OH-D_3-26,23-lactone caused only a slight elevation in serum calcium over the first 24 h of assay. This was followed over the 48–96 h interval by a significant reduction in the serum calcium levels. However, 1α,25-$(OH)_2D_3$ and 25-OH-D_3 generate a significant rise in serum Ca^{2+} levels, in accord with [18,19], demonstrating potent actions of these *seco*-steroids on bone calcium mobilization.

Table 1 shows the dose–response relationship between 25-OH-D_3-26,23-lactone and intestinal calcium transport and bone calcium mobilization. The intestinal calcium transport of 1α,25-$(OH)_2D_3$ reached a maximum within 8 h after dosing, while that of 25-OH-D_3-26,23-lactone only reached a maximum 24 h after administration (fig.1). The more 25-OH-D_3-26,23-lactone is administered, the more intestinal calcium transport is stimulated. The potency of 25-OH-D_3-26,23-lactone in intestinal calcium transport was estimated to be ~120-times less than that of 1α,25-$(OH)_2D_3$. Graded doses of 25-OH-D_3-26,23-lactone had no influence on bone calcium mobilization 24 h after dosing.

Table 2 illustrates the effect of prior dosing with 25-OH-D_3-26,23-lactone on the biological response to a subsequently administered dose of 1,25-$(OH)_2D_3$. In agreement with the results in fig.1,2, 95 h after the administration of the 25-OH-D_3-26,23-lactone alone, there was apparent a significant inhibition of bone calcium mobilization. Intriguingly, the prior administration of 25-OH-D_3-26,23-lactone completely blocked the characteristic bone calcium mobilization response of 1,25-$(OH)_2$-D_3, while it had no inhibiting effect on the intestinal calcium transport of 1α,25-$(OH)_2D_3$.

Table 1
Dose–response relationship between 25-OH-D_3-26,23-lactone and intestinal calcium transport and bone calcium mobilization in vitamin D-deficient rats on a low calcium diet

Compound	Dose (ng)	Time (h)	$^{45}Ca^{2+}$ serosal/ $^{45}Ca^{2+}$ mucosal	Serum Ca^{2+} (mg/100 ml)
Vehicle		8	1.74 ± 0.16	4.74 ± 0.17
1α,25-$(OH)_2D_3$	10	8	2.48 ± 0.15[a]	4.95 ± 0.18[a]
	50	8	4.20 ± 0.22[c]	5.60 ± 0.18[c]
	100	8	5.06 ± 0.31[c]	5.92 ± 0.20[c]
	250	8	5.33 ± 0.28[c]	6.09 ± 0.22[c]
25-OH-D_3-26,23-lactone	100	24	2.14 ± 0.13[a]	4.80 ± 0.16
	500	24	2.65 ± 0.20[b]	4.70 ± 0.18
	2500	24	3.27 ± 0.24[b]	4.62 ± 0.21

Significantly different from control: [a] $p < 0.05$; [b] $p < 0.01$; [c] $p < 0.001$

After 6 weeks on the vitamin D-deficient, low calcium diet, rats were divided into groups of 3 or 5 and each rat received a single intravenous dose of compound in 0.2 ml 0.2% Triton X-100 solution. Control rats received only vehicle. After 8 or 24 h, the animals were decapitated and intestinal calcium transport and serum calcium concentration were measured as in section 2. Data are expressed as mean ± SEM

4. Discussion

We report the first detailed assessment of the biological activity of the characterized vitamin D_3 metabolite, 25-OH-D_3-26,23-lactone. We have employed biosynthetically produced 25-OH-D_3-26,23-lactone, independently shown to have the stereochemical configuration of 23(*S*) and 25(*R*) [9,10]. These studies in the rat indicate that the naturally occurring 25-OH-D_3-26,23-lactone has <1% (table 1) of the activity of 1,25-$(OH)_2$-D_3 in stimulating intestinal calcium transport and no capability to stimulate bone calcium mobilization (fig.1,2). The incubation of rachitic chicken kidney homogenates with 25-OH-D_3-26,23-lactone is known to produce substantial amounts of 1α-25,-dihydroxyvitamin-D_3-26,23-lactone [1,25-$(OH)_2$-D_3-26,23-lactone] [10,17]. The biological activity of the 25-OH-D_3-26,23-lactone may result from enzymatic conversion of this *seco*-steroid into the 1,25-$(OH)_2$-D_3-26,23-lactone.

Table 2
Effect of 25-OH-D_3-26,23-lactone on intestinal calcium transport and serum calcium concentration of vitamin D-deficient rats

Compound given		Intestinal Ca^{2+} transport	Serum Ca^{2+} (mg/100 ml)
Dose 1	Dose 2	$^{45}Ca^{2+}$ serosal/$^{45}Ca^{2+}$ mucosal	
Vehicle	Vehicle	1.71 ± 0.13	4.84 ± 0.18
25-OH-D_3-26,23-lactone	Vehicle	1.80 ± 0.09	4.14 ± 0.19[a]
Vehicle	1α,25-$(OH)_2D_3$	3.96 ± 0.18[a]	5.82 ± 0.16[a]
25-OH-D_3-26,23-lactone	1α,25-$(OH)_2D_3$	3.91 ± 0.10[a]	4.88 ± 0.20

Significantly different from control: [a] $p < 0.001$

Rats fed a low calcium, vitamin D-deficient diet for 6 weeks into 4 groups of 5 rats. They received the first dose of 500 ng 25-OH-D_3-26,23-lactone dissolved in 0.2 ml 0.2% Triton X-100 solution or vehicle only intravenously. After 80 h they received a second dose of 250 ng 1α,25-$(OH)_2D_3$ dissolved in 0.2 ml 0.2% Trition X-100 solution or vehicle only by the same route; 15 h after the second dose, the animals were killed and intestinal calcium transport activity and serum calcium concentration were measured as in section 2. The data are expressed as the mean ± SEM

The striking ability of the 25-OH-D_3-26,23-lactone to apparently inhibit bone calcium mobilization (fig.2; table 2) are novel and intriguing. Regulation of serum calcium levels in animals is known to be under a multifactorial hormonal control involving parathyroid hormone [PTH], calcitonin and 1,25-$(OH)_2$-D_3 [16]. Thus the observed reduction in serum calcium levels could conceivably result from an inhibition by 25-OH-D_3-26,23-lactone of PTH-mediated bone resorption or alternatively a stimulation of renal tubular calcium excretion or an inhibition of renal tubular calcium absorption. Studies are underway to evaluate these several possibilities.

Also as shown in table 2, the 25-OH-D_3-26,23-lactone has a possible significant anti-vitamin action with respect to blocking the actions of 1,25-$(OH)_2$-D_3 on bone calcium mobilization. No vitamin D analogs or metabolites had been identified which have an anti-vitamin activity for 1,25-$(OH)_2D_3$; several compounds, however, have been reported to block the biological response of vitamin D [20,21]. However, it is possible that this apparent inhibition of 1,25-$(OH)_2D_3$ action is simply a separate action of the 25-OH-D_3-26,23-lactone on perturbing the renal tubular handling of calcium (see above) so that the characteristic 1,25-$(OH)_2$-D_3-mediated elevation in serum calcium is not detectable by this experimental protocol. Future experiments will be required to evaluate how combinations of 1,25-$(OH)_2$-D_3 and 25-OH-D_3-26,23-lactone may regulate serum calcium levels. Only then will it be possible to gain insight into the physiological function of the 25-OH-D_3-26,23-lactone.

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