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Spring Time and Fall Time Effects of 1,25-Dihydroxyvitamin D₃, Cobalt (II) and [CoCl₂(1,25(OH)₂D₃)₄] Complex on Renal and Cerebral Enzymatic Markers in Rats

X. Do. Thanh¹, A-S. Coquin², M. Said¹, M.A. Khan² and G. Bouet²

ABSTRACT

In this work, we studied the chronesthesia of 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃), cobalt (II) chloride and of the complex [CoCl₂(1,25(OH)₂D₃)₄]. The study was carried out in spring and autumn on AP and γ -GT activities in brain and kidney of rats during the rodents' active period.

In rat brain, in both seasons, 1,25(OH)₂D₃ enhanced AP activity by 59 % in spring and 21 % in autumn and γ -GT by 39 and 35 % respectively. Cobalt (II) stimulated AP activity by 34 and 29 % respectively. The complex was mainly active on γ -GT activity (70 and 36 %) showing a synergic effect on γ -GT activity in June.

In rat kidney, during spring, the induction of AP activity for 1,25(OH)₂D₃, cobalt (II) chloride and their complex was, respectively 21, 18 and 12 %. The γ -GT activity was not modified during this period, whereas in autumn, it was inhibited by - 33, - 50 and - 28 %. The AP activity in autumn was not altered.

We conclude that the effects on the two enzymatic markers of the three compounds 1,25(OH)₂D₃, cobalt (II) chloride and [CoCl₂(1,25(OH)₂D₃)₄] are quite different in Spring and Autumn, and this is explained on the basis of chronesthesia.

Abstracting keywords: Rat - Renal - Cerebral - Spring - Fall - AP - γ -GT

INTRODUCTION

Usually in vivo studies of molecules effects do not take into account biological rhythms, chronopharmacology. Reinberg et al (1991), Cambar et al (1992) related many experiments on mammals, such as rodents, men.... about the chronotolerance of some molecules. Moore et al (1987) demonstrated that in men, a well known medicine, the acetylsalicylic acid, caused more damages to gastric mucosa in the morning (at the end of the rest span) than in the evening (at the end of the active span) when orally ingested. In previous studies, we already demon-

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strated in intact rats, nocturnal rodents, that two metabolites of vitamin D₃, 24,25-dihydroxyvitamin D₃ (24,25(OH)₂D₃) and 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃), were more active during night time than during day time on renal and cerebral alkaline phosphatase (AP: E-C. 3.1.3.1.) and γ -glutamyltranspeptidase (γ -GT: E.C. 2.3.2.2.) activities (Do et al, 1994; Darcy et al, 1994; Garcion et al, 1994). These enzymatic activities are well known to be the markers of renal proximal tubules brush border membranes and blood brain barrier endothelial cells. According to Guellaën (1989), γ -GT activity is involved in the metabolism of aminoacids and peptides in cell metabolic detoxification. AP activity is widely distributed in nature (Miki, et al, 1986). One of the most interesting side of AP activity is the fact that it is easily induced in response to various stimuli (Miki et al, 1986). We have also demonstrated that these enzymatic activities presented circadian and annual variations (Do et al, 1992; 1995).

Trying to obtain new analogues of vitamin D₃ with the aim of enhancing secosteroids effects, we have pointed out the presence of metallic complexes with cobalt (II): e.g. [CoCl₂(vitD)₄] and [CoCl₂(vitD)₃]. where vitD is a molecule of vitamin D₃ or of an hydroxylated metabolite (Gadais et al, 1994). On the other hand, the high estimated overall stability constants allow us to obtain stable complexes (Do et al, 1994).

In our continuous investigations about the physiological effects of the vitamin D₃ and its relative hydroxylated derivatives, we describe here the effects of 1,25(OH)₂D₃, cobalt (II) and the complex [CoCl₂(1,25(OH)₂D₃)]₄. The procedure for AP and γ -GT activities have described in our earlier publications Besides the measurement of the enzymatic activities, we have also studied the seasonal variations of these two markers.

MATERIAL AND METHODS

Experimental conditions

Female Sprague – Dawley rats from Iffa – Credo (France) weighing from 180 to 220 g were used. Females were chosen because of their regular weight gain and their smaller standard deviation (SD) in AP activity that with male rats (Iffa Credo, 1986). They had free access to both water and standard diet containing 0,8 % Ca, 0,7 % Pi and 3 IU/g Vitamin D. These animals were placed at room temperature in artificial light from 08.00 h to 20.00 h and in darkness from 20.00 h to 08.00 h for a three-week period. The experiments were carried out during the second day of di-oestral state (Laroche et al 1991).

Four series of six rats were used and each animal received a single intraperitoneally (ip) injection (50 μ l) at 03 h 00. On the first of June, the four series of rats were treated as follow:

- control series: the animals received the vehicle (aqueous NaCl, 9 g./l);
- series 1: cobalt (II) chloride, in the 1:4 molar ratio with 1,25(OH)₂D₃;
- series 2: 10 ng/100 g body weight (b.w.) of 1,25(OH)₂D₃;
- series 3: the complex [CoCl₂(1,25(OH)₂D₃)]₄, its concentration was calculated in order to obtain 10 ng/100 g (b.w.) of hydroxylated derivative.

All the animals were killed three hours after receiving i.p. injection, when enzymatic activities of the chosen markers were at their upper level (Do, 1992). The same protocol was used in autumn (30 th September).

Enzyme assays

Brains and kidneys were removed, treated separately, homogenized in a Tris-HCl buffer (50 mmol/l), pH 7.4, supplemented with saccharose (250 mmol/l) and finally the homogenates were frozen at -20°C.

Alkaline phosphatase activity was determined using 4-nitro phenylphosphate as substrate in 2-amino 2-methyl propanol buffer, pH 10.5, with magnesium sulfate (1 mmol/l) at 30°C (Mathieu et al, 1977). γ -glutamyltranspeptidase activity was analysed with γ -glutamyl nitroanilide and glycylglycine, Tris buffer, pH 8.2, at 30°C (Szasz, 1969).

Proteins content in homogenates was estimated according to the Bradford method (1976) with bovine serum albumine as a standard.

Statistical analysis

Results were expressed in specific activities (mIU/mg protein) as the mean \pm standard deviations of four to six determinations of enzymatic activities (1 mIU represents enzymatic quantity hydrolysing 1 nmol substrate per minute). Statistical significances were assessed by the Mann and Whitney non parametric free sample test with $p \leq 0,05$.

RESULTS

Basic cerebral alkaline phosphatase (AP) activity in June and September was 25.6 ± 4.0 mIU/mg protein and 24.6 ± 3.2 mIU/mg protein, while γ -glutamyltranspeptidase (γ -GT) activity was respectively 5.4 ± 1.2 and 3.7 0.8 mIU/mg protein. These values are quite similar to those previously observed (Do et al, 1995).

By night, three hours after i.p injection in rats, cobalt (II) 25 ng/100 g b.w., stimulated cerebral activity. The increase was 34 % ($p \leq 0.05$) in June, and 29 % ($p \leq 0.05$) in September. γ -GT activity was also enhanced but not significantly (respectively 27 and 8 %).

The vitamin D₃ metabolite (1,25(OH)₂D₃) elevated AP activity after injection

Table 1. Spring and Fall effects of 1,25(OH)₂D₃, cobalt (II) and the complex formed by 1,25(OH)₂D₃-cobalt (II) on cerebral alkaline phosphatase (AP) and -glutamyltranspeptidase (γ-GT) activities in Sprague - Dawley rats.
 1,25(OH)₂D₃ = 10 ng/100 g b.w.
 cobalt (II) = 25 ng/100 g b.w.
 Complex: 1,25(OH)₂D₃ (10 ng/100 g b.w.) - cobalt (II) (25 ng/100 g b.w.)
 (): Animal numbers.
 Injection (i.p) at 03.00 h, sacrifices at 06.00 h.
 Values = means ± std deviations (in mIU/mg protein).

	Controls		Cobalt (II)		1,25 (OH) ₂ D ₃		Complex	
	AP	γ-GT	AP	γ-GT	AP	γ-GT	AP	γ-GT
June	25,64 ± 4 (4)	5,4 ± 1,2 (4)	34,4 ± 4 (6)	6,88 ± 2 (6)	40,8 ± 6 (6)	7,52 ± 1,8 (5)	33,8 ± 3,6 (4)	9,2 ± 2,2 (6)
			↑ 34%*	↑ 27%	↑ 59%*	↑ 39%*	↑ 32%	↑ 70%**
			p < 0,05	NS	p < 0,05	p = 0,05	NS	p = 0,01
September	24,6 ± 3,2 (5)	3,7 ± 0,8 (6)	31,7 ± 4 (4)	4,0 ± 0,28 (5)	29,8 ± 4,4 (6)	5 ± 1,8 (6)	29,8 ± 3,2 (5)	5,04 ± 1 (6)
			↑ 29%*	↑ 8%	↑ 21%*	↑ 35%*	↑ 21%	↑ 36%*
			p < 0,05	NS	p < 0,05	NS	NS	p < 0,05

59 % (p ≤ 0.05) and 21 % (p ≤ 0.05) and cerebral γ-GT activity by 39 % (p ≤ 0.05) and 35 % (NS), in June and September respectively.

The complex was very effective for γ-GT activity three hours after injection, it raised the cerebral enzymatic activity by 70 % p = 0.01. In June and in September, γ-GT activity was significantly, though slightly, stimulated (+36 % p ≤ 0.05). As regards the AP activity, it was also increased, however not in a very significant manner (respectively 32 % and 21 %).

Basic renal enzymatic values in June and September were different as already demonstrated (Do et al, 1995). AP activities were respectively 3798±358 and 2496±338 mIU/mg protein, while γ-GT activities were in values 6968±368 and 4441±964 mIU/mg protein.

In June, cobalt (II), 1,25(OH)₂D₃ and the complex respectively stimulated AP activity by 21 %, 18 % p < 0,05 and 12 % (N.S.) while γ-GT activity was slightly inhibited by - 25 %, - 14 % and - 2.7 % (N.S.).

In September all the three compounds seemed to be quite inactive on AP activity (- 2,4 %, - 22 % and - 5 %), and -GT activity was inhibited (-33 % p < 0,05, -50 %, p = 0,01 and -28 %, p > 0,05).

Table 2. Spring and Fall effects of 1,25(OH)₂D₃, cobalt (II) and the complex formed by 1,25(OH)₂D₃-cobalt (II) on renal alkaline phosphatase (AP) and γ-glutamyl-transpeptidase (γ-GT) activities in Sprague - Dawley rats.
 1,25(OH)₂D₃ = 10 ng/100 g b.w.
 cobalt (II) = 25 ng/100 g b.w.
 complex: 1,25(OH)₂D₃ (10 ng/100 g b.w.) - cobalt (II) (25 ng/100 g b.w.)
 (): Animal numbers.
 Injection (i.p) at 03.00 h, sacrifices at 06.00 h.
 Values = means ± std deviations (in mIU/mg protein)

	Controls		Cobalt (II)		1,25 (OH) ₂ D ₃		Complex	
	AP	γ-GT	AP	γ-GT	AP	γ-GT	AP	γ-GT
June	3798 ± 358 (5)	6968 ± 368 (4)	4610 ± 320 (5)	6789 ± 889 (6)	4498 ± 186 (5)	5972 ± 572 (6)	4252 ± 414 (5)	6778 ± 963 (6)
			↑ 21%*	↓ 2.5%	↑ 18%*	↓ -14%	↑ 12%	↓ 2.7%**
			p < 0,05	NS	p < 0,05	NS	NS	NS
September	2496 ± 338 (5)	4441 ± 964 (5)	2256 ± 300 (6)	2979 ± 586 (5)	1940 ± 192 (6)	2219 ± 305 (5)	2358 ± 270 (6)	3206 ± 505 (5)
			-9,6%	↓ -3,3%	↓ -22%	↓ -50%	↓ -5%	↓ -28%
			NS	*	NS	**	NS	NS
			p < 0,05		p = 0,01			

DISCUSSION

This work showed that in rats the effects of 1,25(OH)₂D₃, cobalt (II) and their complex [CoCl₂(1,25(OH)₂D₃)] were quantitatively different in June and at the end of September (Tables 1 and 2) in animals kept in constant conditions.

In *Ipfa credo* laboratories, Sprague-Dawley rats are kept under artificial light and dark (12/12). So, we maintained our animals under the same conditions. To Cambar et al (1992), Reinberg et al (1991), the experiments under constant conditions (e.g.: light, temperature ...) with laboratory animals during many seasons showed that their seasonal rhythms were not altered. The discovery of *per* gene enhanced the hypothesis that most biological rhythms are genetically programmed.

Many studies were undertaken showing circadian chronesthesia of various drugs: corticoids are well supported by asthmatic people and are more effective when administered from 08.00 h to 14.00 h, than in the afternoon from 15.00 h to 20.00 h (Gervais et al, 1987). Theophyllin, injected in rats at 08.00 h, did not

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