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Day time and night time effects of 24R,25-dihydroxycholecalciferol on renal alkaline phosphatase and gamma-glutamyltransferase activities in intact rats

BY

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The effects of 24R,25-dihydroxycholecalciferol [24,25(OH)₂ CC] on alkaline phosphatase (APA), gamma-glutamyltransferase (γ-GT) and acid phosphatase (AP) activities were investigated on renal cortex slices of intact rats killed at 18h00 or at 06h00. At 06h00, three, six and nine hours after a single intraperitoneal injection of 24,25(OH)₂ CC, APA activity was increased by 30%, 51% and 29%, respectively and γ-GT activity, by 25%, 39% and 38%, as compared to their controls. AP on the other hand was not modified at all. These enzymatic stimulations were statistically higher than those measured at 18h00, respectively APA : 11%, 25%, 28% and γ-GT : 2%, 20%, 22%. They can be explained by variations in new protein synthesis in relation with rat activity periods. Physiological significance of these renal effects remains to be elaborated.

Key Words : rat; kidney; alkaline phosphatase; acid phosphatase; gamma-glutamyltransferase.

Introduction

Vitamin D₃ or cholecalciferol (CC) was converted predominantly in the kidney into 1 α, 25-dihydroxycholecalciferol [1,25(OH)₂ CC] and 24 R, 25-dihydroxycholecalciferol [24,25(OH)₂ CC]. Although 1,25(OH)₂ CC is considered to be the active form of CC, there is evidence that 24,25(OH)₂ CC possesses its own physiological role. This was demonstrated by stimulation of alkaline phosphatase (APA) on rat endometrial cells. (LIEBERHERR *et al.*, 1984), on rat calcitonin stimulated cyclase in bone (GORDELADZE & GAUTVIK, 1986). For LIDOR *et al.* (1987), the secosteroid administered locally to D-deficient chicks healed rachitic lesions in legs only.

One of the continuing goals of our laboratories is the study in rats of the effects of 24,25(OH)₂ CC on renal alkaline phosphatase (APA : E.C. 3.1.3.1.), gamma-glutamyltransferase (γ-GT : E.C. 2.3.2.2) and acid phosphatase (AP : E.C. 3.1.3.2.) activities. We already demonstrated that *in vitro* the metabolite increases significantly APA and γ-GT activities after 6 hours incubation with 1 nmol/l of 24,25(OH)₂ CC in hypophysectomized (Hx) rat kidneys. AP was not modified. *In vivo* APA and γ-GT were also enhanced by the secosteroid 6 hours after intraperitoneal (ip) injection. (DO THANH *et al.*, 1988, 1991, 1993; PRELOT *et al.*, 1991).

These conventional experiments did not take into account rat biological rhythms. Some authors (REINBERG, 1989; LEVI *et al.*, 1989) have reported that

mice and rats rest during day and are active during dark period. They are more sensitive to drugs in the dark span as compared to the light span. It was demonstrated also that there is circadian rhythm in γ-GT and leucine aminopeptidase urinary activity in rats (CAL *et al.*, 1993).

In a previous study, we showed that in intact rats values of renal APA and AP activities are much higher by night than those found during daytime; γ-GT on the other hand does not undergo any modification by night (DO THANH *et al.*, 1992) probably because of maximal urinary excretion of this enzyme occurring at the end of the rest span (CAL *et al.*, 1993).

The aim of the present study was to ascertain whether the vitamin D₃ metabolite 24,25(OH)₂CC activity was the same in night and day-times.

Material and Methods

Animals

Female Sprague-Dawley rats from Iffa-Credo (France) weighing from 160 to 200 g were used. 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 in artificial light from 08h00 to 20h00 and in darkness from 20h00 to 08h00 for a period of three weeks, at room temperature.

1 - Nine series of 6 rats received intraperitoneally (ip) a single dose of 5 ng/100 g body weight 24,25(OH)₂ CC dissolved in ethanol-propanediol (vol/vol), the same volume of this vehicle or no treatment at all. After

TABLE III. — In vivo diurnal and nocturnal effects of Actinomycin D, 24,25 (OH)₂ CC, Actinomycin D and 24,25 (OH)₂ D₃ on renal alkaline phosphatase and gamma-glutamyltransferase in intact rats.

Treatment (Injections made at)	Animal number	Specific activities (mU/mg proteins)		
		APA	γ-GT	AP
<i>Diurnal experiments</i>				
12h00				
Controls	6	1899 ± 312	4174 ± 222	69 ± 18
24,25(OH) ₂ CC	6	2374 ± 360**	5009 ± 410**	71 ± 20
Actinomycin D	6	1915 ± 492	4082 ± 053	70 ± 15
Act D + 24,25	6	1736 ± 421	4222 ± 464	68 ± 13
<i>Nocturnal experiments</i>				
24h00				
Controls	6	4005 ± 545	4319 ± 563	98 ± 13
24,25(OH) ₂ CC	6	6017 ± 436**	6003 ± 414**	101 ± 16
Actinomycin D	6	3962 ± 477	4300 ± 629	100 ± 18
Act D + 24,25	6	4110 ± 508	4279 ± 544	99 ± 15

Female Sprague-Dawley rats received a single intraperitoneal (ip) injection of different molecules (Actinomycin D : 20 µg/100 b.w.; 24,25(OH)₂ CC : 5 ng/100 g b.w.; Actinomycin D : 20 µg/100 g b.w. - mixed with 24,25(OH)₂ CC (5 ng/100 g b.w.))

Animals were killed 3, 6 and 9 hours after administration.

Values are means ± standard deviation.

For diurnal experiments, molecules were injected at 12h00, kidneys were removed after cervical dislocation at 18h00.

For nocturnal experiments, molecules were injected at 24h00, kidneys were removed after cervical dislocation at 06h00.

Mann-Whitney non parametric test was used : * $P < 0.01$;

N.B. : Actinomycin D mixed with 24,25(OH)₂ CC did not show any artifactual effect in our in vitro experiments (PRELOT *et al.*, 1991).

2 - Nine series of rats were prepared in the same way. They were killed at 06h00, after drug or vehicle administration at 03h00, 24h00, and 21h00 (night series).

3 - Four series of 6 rats received intraperitoneally 5 ng/100 g b.w. 24,25(OH)₂ CC, vehicle, actinomycin D alone (20 µg/100 g b.w.) and actinomycin D mixed with 24,25 (OH)₂ CC at 12h00. They were killed at 18h00. The same experiment was repeated with rats injected at 24h00 and killed at 06h00.

Assays

Kidneys were removed immediately and renal cortex were minced and homogenized in a solution containing saccharose (250 mmol/l), Tris-HCl (50 mmol/l), pH 7.4.

APA and AP were determined using nitro-4 phenylphosphate as substrate in amino-2-methylpropanol buffer, pH 10.5 at 30°C (MATHIEU *et al.*, 1977) and in citrate buffer, pH 4.8 at 37°C (ANDERSCH *et al.*, 1947).

γ-GT was analyzed with γ-glutamyl-nitroanilide and glycylglycine, pH 8.2 at 30°C (SZASZ, 1969).

BRADFORD method (1976) was used for protein determinations with bovine serum albumin as standard.

Statistics

The results are given as mean of triplicate values of enzymatic activities ± standard deviation. Statistical significance are assessed by the Mann-Whitney non parametric free sample test, with $P \leq 0.05$.

Results

To avoid experimental errors, each treatment time had its own control.

Preliminary experiments showed that there were no differences between basic enzymatic activities from kidneys of rats receiving vehicle in i.p. and kidneys of rats without any injection (Tables I and II).

1 - For animals without treatment killed at 06h00 and at 18h00, the enzymatic activities of APA and AP were significantly increased. They are grouped in Table I.

2 - In the day-series, three, six and nine hours after a single ip injection of 24,25(OH)₂ CC, APA activity was increased respectively by 10% (NS), 25% ($P < 0.05$) and 28% ($P < 0.05$) as compared to controls (Table II).

γ-GT activity was stimulated only after six and nine hours by 20% ($P < 0.05$) and 22% ($P < 0.05$); AP activity was not modified (Table II).

3 - In the night series APA and AP basal activities were much higher than diurnal basal activities (+ 113%, $P < 0.01$ for APA and + 42%, $P < 0.05$ for AP) but γ-GT activity was not modified (Table II), which confirmed our previous results. (Do *et al.*, 1992).

By night a single ip injection of 24,25(OH)₂ CC (5 ng/100 g b.w.) was made at 03h00, 24h00 and 21h00. APA activity was enhanced respectively by 30% ($P \leq 0.01$), 51% ($P < 0.01$) and 29% ($P < 0.01$), γ-GT activity by 25% ($P < 0.01$), 39% ($P < 0.01$) and 38% ($P < 0.01$).

4 - APA and γ-GT enhancements were suppressed by actinomycin D, a well known transcriptional inhibitor (Table III). AP activity was not modified (Table II).

Discussion and Conclusion

We confirm that intact rats are sensitive to renal effects of 24,25(OH)₂ CC. Actual finding corroborate

our previous studies in hypophysectomized rats (Do *et al.*, 1988, 1991, 1993).

REINBERG (1989), LEVI *et al.* (1989) as well as CAL *et al.* (1993), have recommended that biological rhythms of animals must be respected in order to avoid some misleading conclusions. In fact, the results obtained with rats killed at 06h00 showed that nocturnal life influenced greatly the enzymatic renal activities. The differences were quantitative.

Stimulations of APA activity by 24,25(OH)₂ CC were higher by night than by day when compared to their respective controls (30%, 51% and 29% vs 16%, 25% and 28%). However nocturnal increases of APA activity 9 hours after ip injection were quite identical to diurnal enzymatic activity (29% vs 28%): this may be due to a more rapid metabolism of the drug during the nocturnal activity period.

Nocturnal stimulations of γ -GT activity by the secosteroid were faster (3 h), higher in values (39% vs 22%) and of longer duration (more than 9 hours) than those obtained during the day span: it seems that γ -GT activity is more sensitive by night to this low dose of 24,25(OH)₂ CC. AP activity was not significantly altered.

The latency of 24,25(OH)₂ CC to stimulate APA and γ -GT activities, is about three hours after secosteroid injection, which suggests protein synthesis. This hypothesis is supported by the fact that actinomycin D inhibits these renal effects during night as well as during day (Table III). According to GLOSSMANN and NEVILLE (1972), APA was studied extensively but little is known about its precise physiological role, while γ -GT catalyzed the transfer of the γ -glutamyl moiety of glutathione into a variety of aminoacids and peptides. Both enzymatic activities are known in rats to be highly specific for kidney brush border membrane. (BRAUN *et al.*, 1978). The physiological significance of these renal effects remain to be clarified by further experiments.

It can be concluded that during the nocturnal active life of rats, the renal APA and γ -GT activities are increased. They are more sensitive to the effects of 24,25(OH)₂ CC. These enzymatic stimulations are

specific. They are probably due to new protein synthesis.

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References

- ANDERSCH, M.A. & SZCZYPINSKI, A.J. (1947) *Am. J. Clin. Path.* 17, 571-574.
- BRADFORD, M.M. (1976) *Anal. Biochem.* 72, 248-254.
- BRAUN, J.P., RICO, A.G., BENARD, P., BURGAT-SACAZE, V., EGHBALI, B. & GODFRAIN, J.C. (1978) *Toxicology* 11, 73-82.
- CAL, J.C., LEMOIGNE, F., CROCKETT, R. & CAMBAR, J. (1993) *Chronobiology Int.* 4, 153-160.
- DO THANH, X., BOISNARD, P., GIRAULT, A., PLANCHENAU, P., BREGET, R. & PRELOT, M. (1992) *Sci. Tech. Anim. Lab.* 14, 207-211.
- DO THANH, X., GIRAULT, A. & PRELOT, M. (1988) *J. Physiol. (London)* 406, 133.
- DO THANH, X., GIRAULT, A. & PRELOT, M. (1993) *Arch. Int. Physiol. Biochim. Biophys.* 101, 367-369.
- DO THANH, X., PRELOT, M., PLANCHENAU, P. & GIRAULT, A. (1991) In: Vitamin D: Gene Regulation, Structure - Function Analysis and Clinical Application, 310-311. Ed. NORMAN A.W., BOUILLON, R., THOMASSET, M., W. DE GRUYTER Berlin, New York.
- GLOSSMANN, H. & NEVILLE, D.M. JR (1972) *FEBS Letters* 19, 340-344.
- GORDELADZE, J.O. & GAUTVIK, K.M. (1986) *Biochem. Pharmacol.* 35, 899-902.
- LEVI, F., BOUGHATTAS, N.A. & BLAZSEK, I. (1989) In: Ann. Rev. Chronopharmacol. 4, 283-331. Ed. Pergamon, Oxford.
- LIDOR, C., ATKIN, I., ORNOY, A., DEKEL, S. & EDELSTEIN, S. (1987) *J. Bone Miner. Res.* 2, 91-98.
- LIEBERHERR, M., ACKER, G.M., GROSSE, B., PESTY, A. & BALSAN, S. (1984) *Endocrinology* 115, 824-829.
- MATHIEU, M., BRETAUDIERE, J.P., GALTEAU, M., GUIDOLLET, J., LALEGRIE, P., BAILLY, M., BORET, P., DORCHE, C., LOUISOT, P. & SCHIELE, F. (1977) *Ann. Biol. Clin.* 35, 271-273.
- PRELOT, M., DO, T.X., GIRAULT, A. & THUILLIER, A. (1991) *Arch. Int. Physiol. Biophys.* 99, 269-273.
- REINBERG, A. (1989) *Sci. Tech. Anim. Lab.* 14, 85-89.
- SZASZ, G. (1969) *Clin. Chemistry.* 15, 124-136.

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