Copper(II) and 24,25-(OH)2D3-copper(II) complex inhibit circadian effects of 24,25-(OH)2D3 on hypophysectomized rats APA sera activity.

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COPPER (II) AND 24,25-$(OH)_2D_3$-COPPER (II) COMPLEX INHIBIT CIRCADIAN EFFECTS OF 24,25-$(OH)_2D_3$ ON HYPOPHYSECTOMIZED RATS APA SERA ACTIVITY

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Introduction

It has already been reported that the metabolism of Vitamin D_3 in hypophysectomized (Hx) rats is disturbed : sera $1\alpha,25(OH)_2D_3$ decreases while $24,25(OH)_2D_3$ production is slightly increased (1). Alkaline phosphatase (APA) activity is depressed (2). DO *et al.* (3, 4) have demonstrated that intraperitoneal (ip) injections of $24,25(OH)_2D_3$ induce an increase in APA and γ-glutamyl-transferase (GGT) activities in rats brain and kidneys. It interesting to investigate whether these enzymatic activities could also be modified in sera of Hx rats.

Materials and Methods

Intact and Hx male Sprague-Dawley rats from Iffa-Credo (France) weighing, on receiving day, from 180-220 g were used.Five series of 4 rats were used and each animal received a single intraperitoneal (ip) injection (50 µl) at 03.00 h. Treatments were as follow:

- control series: animals received vehicle (aqueous NaCl 9 $g.l^{-1}$)
- series 1 : copper (II) chloride ($4.8.10^{-7}$ $mol.l^{-1}$)
- series 2 : $24,25(OH)_2D_3$ ($4.8\ 10^{-7}$ $mol.l^{-1}$)
- series 3 : $[CuCl_2(24,25\ (OH)_2\ D_3)]$ complex ($4.8\ 10^{-7}$ $mol.l^{-1}$)

-intact series: intact animals received vehicle (aqueous NaCl 9g l-1)

At night time all animals were killed 3 h after ip, when enzymatic activities were at their highest levels (5). The same protocol was used at day time (ip at 15.00 h). APA (Enzyline kit®, Biomerieux) and GGT (GGT kit®, Boehringer) activities were tested in sera.

Results and Discussion

In intact and in Hx rats values of sera APA activity were lower during day time than night time (Tables I & II). Hypophysectomy depresses APA values when compared to intact rats (- 43 % $p < 0.05$). Sera GGT activity could not be detected (6). During day, 3 h after injection $24,25(OH)_2D_3$ inhibits APA activity in Hx rats (- 26% $p < 0.05$). At night time $24,25(OH)_2D_3$ is more potent on sera APA activity as compared to day time (- 48 % vs -26 % $p < 0.05$).

These results are consistent with previous study demonstrating that 24,25 $(OH)_2\ D_3$ inhibits directly parathyroïd hormone (PTH) action on bone through abolishment of PTH-stimulated adenylate cyclase activity (7). Copper (II) and the complex suppress secosteroid effects, it's more effective at night time than during day time. These data prove that rats are more sensitive to the effects of $24,25(OH)_2D_3$ on sera APA activity during night than during day. Copper (II) and

the complex abolish secosteroid effects and this can be explained on the basis of circadian chronopharmacology.

Table I - Day time effects of $24,25(OH)_2D_3$, copper(II) and $24,25(OH)_2D_3$-copper(II) complex on sera (APA) activity (mU/ml, means±s.d.) in Hx rats 3 h after i.p. injection (15.00 h) and sacrifice (18.00h), Mann-Whitney test with $p < 0.05$.

Intact rats a	Hypophysectomized rats			
	Controls [b]	$24,25(OH)_2D_3$ [c]	Copper(II) [d]	Complex [e]
207±28	145±23, a ≠ b -30 % p<0.05	107±10, b ≠ c -26 % p<0.05	133±25, b ≠ d - 8 % n.s.	147±39, b ≠ e n.s.

Table II - Night time effects of $24,25(OH)_2D_3$, copper(II) and $24,25(OH)_2D_3$-copper(II) complex on sera (APA) activity.

Intact rats aa	Hypophysectomized rats			
	Controls [bb]	$24,25(OH)_2\ D_3$ [cc]	Copper(II) [dd]	Complex [ee]
728±84	387±126, aa≠bb -47 % p<0.05	107±10 bb≠cc -48 % p<0.05	133±25 bb≠dd - 5 % n.s.	147±39 bb≠ee n.s.
	aa≠bb, 72% p<0.05			

a ≠ aa : + 71 %, $p<0.05$ b ≠ bb : + 62 %, $p >0.05$ c ≠ cc : +47 %, $p<0.05$

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