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Respiratory Alkalosis and Reduced Plasmatic Concentration of Ionized Calcium in Rats Treated with 1,25 Dihydroxycholecalciferol

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Summary. The daily administration of supraphysiological doses of 1,25 dihydroxycholecalciferol (0.1–2.5 μg/d/100 g body weight) to rats, produced respiratory alkalosis. With the doses of 0.1–0.2 μg/d/100 g and feeding a diet with 0.7% of calcium, calcemias did not exceed 2.75 mM, and significantly reduced plasma ionized calcium levels were measured. The latter phenomenon was found associated with increased urinary excretion of cAMP, soft tissue calcium content, and polyuria with hyposthenuria, all known effects of parathyroid hormone. These effects were absent in thyroparathyroidectomized rats treated in the same fashion. Present results suggest that the stimulus of low levels of plasma ionized calcium overcomes the probably inhibitory effect of the steroid on parathyroid hormone secretion.

Key words: Respiratory alkalosis — Ionized calcium — 1,25 Dihydroxycholecalciferol — Parathyroid hormone

This paper describes the development and metabolic consequences of the respiratory alkalosis produced in rats by the administration of supraphysiological doses of 1,25 dihydroxycholecalciferol ($1,25(OH)_2D_3$). With adequate doses and calcium intakes, calcemia did not raise above 2.75 mM and then, a significant reduction in plasma ionized calcium concentration was observed. Indirect evidence obtained with intact and thyroparathyroidectomized (TPTX) animals confirmed the expected stimulation of parathyroid hormone (PTH) secretion.

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Material and Methods

Inbred IIM rats (sub-line "m"), weighing 150–200 g were housed in individual metabolic cages and fed the standard laboratory diet (Ca 0.7%, P 0.6%). Thyroparathyroidectomies were performed under ether anesthesia, and those animals with calcemias above 2.0 mM were discarded. TPTX rats received 2 μg/d of *l*-thyroxine.

For 10 days unless otherwise stated, treated animals received daily (between 8–9 A.M.) oral doses of $1,25(OH)_2D_3$ ranging from 0.05–2.5 μg/d/100 g bw, in 0.1 ml of water. Control animals received solvent alone.

Unless otherwise stated, the animals were sacrificed 24 h after the last dose. Blood was drawn by heart puncture under light ether anesthesia, to measure pH, pO_2 and P_{CO2} in a Radiometer instrument, ionized calcium with a flow-through specific electrode (Orion Biomedical SS-20), plasma calcium [9], phosphate [3], and citrate [12]. Respiratory frequency was determined visually with the aid of a chronometer during the intermittent sleeping periods in a room in which the animals were undisturbed.

The effect of $1,25(OH)_2D_3$ administration on PTH secretion was assessed through a 2 × 2 experimental design: intact and TPTX rats were divided into control and treated animals (0.2 μg of the steroid/d/100 g bw) as indicated above.

The urinary excretion of cAMP [6] was measured in the 6 h following the administration of the steroid; the results were expressed in nmoles of cAMP/h. Urine was collected daily during the experimental period, its osmolality measured in an Advanced Instrument Osmometer and the data averaged for each rat. At the end of the experiments, both kidneys were excised, weighed, incinerated, and the ashes dissolved in N HCl to measure calcium [17].

Results

The administration of $1,25(OH)_2D_3$ at the rate of 0.10, 0.20, 0.50, and 1.25 μg/d/100 g bw (6 rats per

Table 1. Acid-base status and respiratory frequency of control and $1,25(OH)_2D_3$-treated rats

	Controls n = 9	$1,25(OH)_2D_3$[a] n = 24
H^+ nEq/l	47.8 ± 0.7	40.7 ± 1.0[d]
pH	7.32 ± 0.02	7.39 ± 0.02[d]
P_{CO_2} mm Hg	42.6 ± 2.0	36.6 ± 2.0[c]
CO_3H^- mM	22.2 ± 0.8	21.3 ± 1.2
pO_2 mm Hg	60.4 ± 4.2	50.0 ± 3.0[b]
Respiratory frequency, min^{-1}	82 ± 1	95 ± 1[d]

[a] Four groups of 6 rats each treated with 0.1, 0.2, 0.5, and 1.25 μg of $1,25(OH)_2D_3$/d/100 g bw, for 10 days
[b,c,] and [d] Significant differences at the 0.05, 0.01, and 0.001 levels. The figures indicate the mean ± standard error

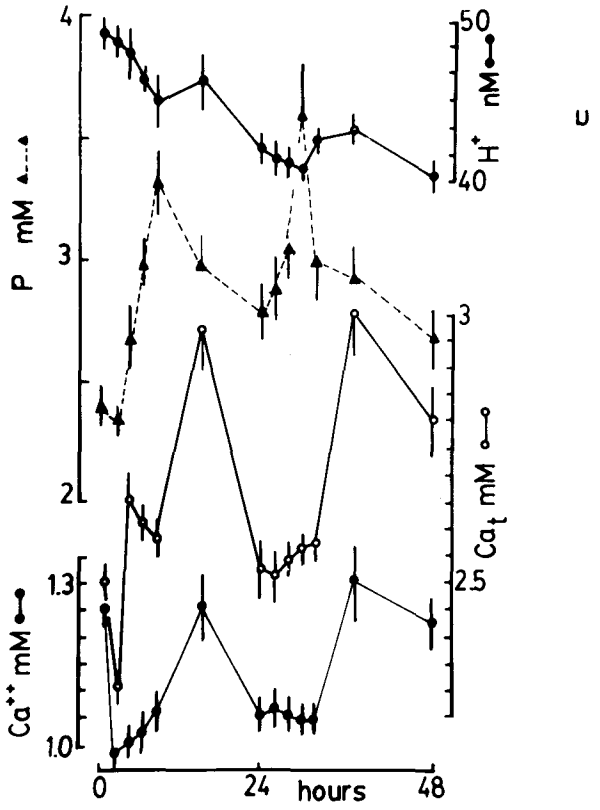

Fig. 1. Time course study of the plasma levels of calcium (total and ionized) and phosphate and blood concentration of hydrogen ion after two doses of 0.2 μg of $1,25(OH)_2D_3$/100 g body weight, administered per os at the beginning of the experiment and 24 h later. The symbols indicate the mean ± standard error (4–6 per point).

dose level) for 10 days, produced, without differences between doses, a significant increase in blood pH due to decreased P_{CO2} (Table 1). The increased respiratory frequency confirmed the diagnosis of respiratory alkalosis.

Figure 1 presents the modifications of the plasmatic concentrations of H^+, phosphate, and calcium (total and ionized), following the administration of 0.2 μg/d/100 g bw of the steroid at the beginning of the experiment and 24 h later. Calcemia and phosphatemia showed peaks at 12–16 and 6–8 h, respectively after the oral dose of the hormone. Blood pH decreased steadily and as a consequence, at all sampling where calcemia did not exceed 2.75 mM, the concentration of ionized calcium was significantly reduced in respect to control levels ($P < 0.001$). The effects of a lower dose (0.1 μg/d/100 g) on the ionized calcium concentration needed 48 h (2 doses) to develop. A still lower dose (0.05 μg/d/100 g) had no significant effect (Fig. 2). After feeding of a calcium-enriched diet (Ca 1.6%), the dose of 0.2 μg/d/100 g produced calcemias above 2.75 mM, and higher than normal levels of ionized calcium were measured.

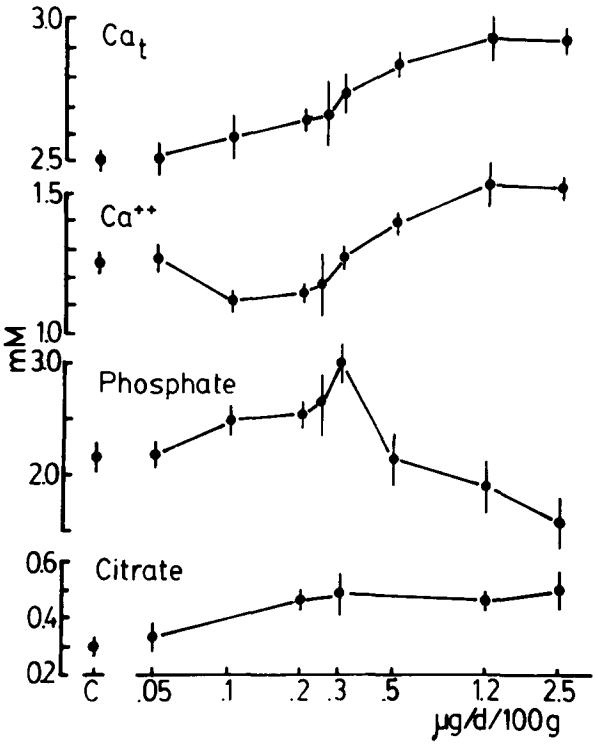

Fig. 2. Plasmatic concentrations of calcium (total and ionized), phosphate, and citrate of controls (C) and of rats treated with the indicated doses of $1,25(OH)_2D_3$. Each point indicates the mean ± standard error of 6 animals.

Figure 2 presents the plasmatic concentrations of total and ionized calcium, phosphate, and citrate after 10 doses of $1,25(OH)_2D_3$ administered at daily intervals. With modest doses (0.1–0.2 μg/d/100 g), a significantly decreased Ca^{2+} was observed ($P < 0.001$) that could be explained by the concurrent alkalosis, though some participation of the increased concentrations of phosphate and citrate cannot be excluded.

In the next 6 h following the administration of 0.2 μg/100 g of $1,24(OH)_2D_3$, the urinary excretion of cAMP increased significantly (Table 2). Treated animals had polyuria without significant differences in the excretion of total solutes. After 10 days of treatment, the renal calcium content was significantly increased. The three mentioned effects were absent in treated TPTX rats though increased blood pH (controls 7.39 ± 0.02, n = 12; treated 7.44 ± 0.02, n = 10, $P < 0.01$) and reduced ionized calcium levels (controls 2.15 ± 0.21 mEq/liter, n = 12, treated 1.91 ± 0.12, n = 10, $P < 0.001$) were produced as expected.

Discussion

A previous report from this laboratory [10] indicated that the administration of supraphysiological doses of $1,25(OH)_2D_3$ to rats, produced a disturbance of the P metabolism of the red cells that resulted in tissue hypoxia, as assessed by the increased levels of erythropoietin and lactate/pyruvate ratio. That phenomenon may be related to increased respiratory frequency and its corollary, respiratory alkalosis. Administration of modest doses of the steroid and feeding a diet with 0.7% of calcium, produced calcemias that did not raise above 2.75 mM, and a significant reduction in the plasmatic concentration of ionized calcium was observed. The phenomena reported in this paper bear no relationship with the metabolic alkalosis reported for some patients intoxicated with vitamin D [16, 18].

The time course study of the changes in several plasmatic components, produced by the administration of 0.2 μg/d/100 g bw of the hormone, showed a periodic elevation of phosphatemia and calcemia. The former, with a peak 6–8 h after dosing, is in agreement with the kinetic of erythrocyte 2,3-diphosphoglycerate hydrolysis [10]. The peak of calcemia (16–18 h after dosing) appears to be the result of bone resorption produced by the steroid itself [15] and/or the assumed increased levels of PTH (see below). According to Haussler et al. [8] and Norman et al. [13], the enhancement of intestinal calcium absorption shows a maximum 8–10 h after $1,25(OH)_2D_3$ administration.

The following observations are consistent with the stimulation of PTH secretion by the decreased Ca^{2+} levels, in spite of the assumed increased levels of the steroid: (a) the urinary excretion of cAMP increased [2] in coincidence with the fall of Ca^{2+} in plasma; (b) the calcium content of kidney tissue was significantly increased [1, 5, 7]; (c) polyuria with hyposthenuria [4, 5] was produced as a consequence of treatment; and (d) the mentioned effects could not be reproduced in TPTX animals.

In agreement with present results, it is worth pointing out that after a single supraphysiological dose of $1,25(OH)_2D_3$ to normal puppies, Oldham et al. [14] found a significant peak of serum iPTH 1 h after dosing, and hyperphosphatemia by the sixth hour. Probably because the animals were fasting, no significant effect was observed in total serum calcium within 12 h after administration of the steroid.

The mutual regulation between the two calcemic hormones, PTH and $1,25(OH)_2D_3$, has been defined by other investigators in terms of two negative feedback loops: a direct inhibition (short loop) of PTH secretion by the steroid itself [11 and reviewed literature] and an indirect long loop via the concentration of Ca^{2+} in plasma. Present results suggest that the stimulus of low Ca^{2+} levels overcomes the direct inhibitory effect of the steroid on PTH secretion.

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Table 2. Urinary cAMP excretion, kidney Ca content, and diuresis and its osmolality of intact and thyroparathyroidectomized, control and treated (0.2 μg/d/100 g) rats

	cAMP nmol/h	Kidney Ca μmol/g	Diuresis ml/d	Osmolality mOsm/kg
Intact rats (n = 8)	1.70 ± 0.19	4.9 ± 0.5	7.2 ± 0.5	2421 ± 130
Intact + $1,25(OH)_2D_3$ (n = 10)	2.78 ± 0.51[b]	43.7 ± 4.9[c]	19.2 ± 0.5[c]	1161 ± 136[c]
TPTX rats (n = 10)	0.93 ± 0.18	4.0 ± 1.0	5.6 ± 1.3	1713 ± 105
TPTX + $1,25(OH)_2D_3$ (n = 10)	0.70 ± 0.08	7.7 ± 1.8	5.1 ± 0.4	1801 ± 126

[b] and [c] Significant differences at the 0.01 and 0.001 levels. The figures indicate the mean ± standard error

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