THE FUNCTIONAL METABOLISM OF VITAMIN D IN RATS TREATED WITH CORTISOL

S. EDELSTEIN and D. NOFF
Biochemistry Department, The Weizmann Institute of Science, Rehovot

and

A. MATITIAHU, R. SAPIR and A. HARELL
Department of Endocrinology, Municipal-Governmental Medical Centre Ichilov-Hospital and Sackler School of Medicine, Tel-Aviv University, Tel-Aviv-Jaffo, Israel

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1. Introduction

Glucocorticoids are known as vitamin D antagonists. Cortisol is used in the treatment of hypercalcemia due to vitamin D intoxication [1], sarcoidosis [2] or idiopathic hypercalcemia of infancy [3].

Plasma calcium homeostasis is controlled by the interaction of the intestine, the kidney and the bone. These tissues are also thought to be target organs for vitamin D action, and the functional form of the vitamin in these sites is believed to be the dihydroxylated derivative, $1,25(OH)_2D_3$*. This compound is produced from vitamin D by C-25 hydroxylation in the liver, followed by C-1 hydroxylation in the kidney. When $1,25(OH)_2D_3$ interacts with intestinal or kidney cells, a specific $CaBP^+$ is formed [4–6], which is believed to be involved in the process of calcium transport.

Animal studies have shown that glucocorticoids are linked with decreased serum calcium concentration and decreased intestinal transport and absorption of calcium [7,8]. The present study was undertaken in order to establish whether the hypocalcenic effect of glucocorticoids is mediated via an effect on the metabolism of vitamin D, and the subsequent biological expression of the vitamin in the form of CaBP.

It was established, that cortisol treatment in rats is associated with decreased accumulation of $1,25(OH)_2D_3$ in both intestinal mucosa cells and kidney cells, with subsequent decrease in the concentration of the vitamin D-dependent intestinal CaBP.

Abbreviations: $25(OH)D_3$, 25-hydroxycholecalciferol; $24,25(OH)_2D_3$, 24,25-dihydroxycholecalciferol; $1,25(OH)_2D_3$, 1,25-dihydroxycholecalciferol; CaBP, calcium-binding protein

Correspondence to: Samuel Edelstein, Biochemistry Department, The Weizmann Institute of Science, Rehovot, Israel

2. Experimental

2.1. *Animals*

Young male rats (Wistar) weighing 25–30 g were maintained for four weeks on a vitamin D-deficient diet [9], modified for rats as previously described [10]. At the end of this depletion period a four-week repletion period with radioactively labelled cholecalciferol started while the rats were being fed the same vitamin D-deficient diet. Every five to seven days during this period, each rat received a subcutaneous injection of 0.75 μg [1,2-^{3}H, 4-^{14}C]cholecalciferol dissolved in 0.1 ml propylene glycol. At the end of the repletion period the rats were divided into two groups, and for an additional 8 days were injected daily, respectively with: (a) propyleneglycol; (b) cortisol. Two days after the last injection the rats were killed, and tissues were taken for analysis.

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2.2. *Analysis of the metabolites of cholecalciferol*

After killing, the scraped mucosa from the proximal 12 cm of the small intestine and the kidneys of six rats from each group were removed weighed, minced, pooled and extracted for lipids [11]. The lipid extracts were analyzed for cholecalciferol metabolites using thin-layer chromatography (t.l.c.) [12]. The relative amount of $1.25(OH)_2D_3$ in the polar peaks eluted from the t.l.c. plates was calculated from the $^3H/^{14}C$ specific radioactivity ratios [13].

2.3. *Analysis of intestinal CaBP*

Rats of comparable groups, which were injected with non-radioactive labelled cholecalciferol were killed, and the proximal 12 cm of the small intestine were removed. The mucosa of six such segments for each group was scraped, pooled, harvested and processed for analysis of CaBP content as previously described [14].

2.4. *Materials and doses*

[1,2-^{3}H]cholecalciferol (spec. radioactivity 12.6 Ci/mmol) and [4-^{14}C]cholecalciferol (spec. radioactivity 32.3 Ci/mol) were obtained from the Radiochemical Centre, Amersham, Bucks, England. [1,2-^{3}H, 4-^{14}C]Cholecalciferol was prepared by mixing the two single-labelled preparations so as to give a $^3H/^{14}C$ ratio of approx. 5.0. Cortisol (Δ^4-pregnen-11β,17α, 21-triol-3, 20-dione) was obtained from Sigma Chemical, St. Louis, Mo. USA. Each rat received a daily subcutaneous injection of 1 mg cortisol, dissolved in 0.1 ml propylene–glycol.

2.5. *Measurement of radioactivity*

All radioactivity measurements were carried out in a Packard TriCarb automatic counter. Quenching was corrected for by the automatic external standardization and correlation curves for combined ^{14}C and ^3H.

3. Results

Since chemical methods for measuring cholecalciferol metabolites are not readily available, the procedure of long-term administration of double-labelled radioactive cholecalciferol to rats employed in the present study, enabled the measurement of all the metabolites of cholecalciferol in target tissues for vitamin D action. Under these conditions, cortisol treatment was found to result in decreased accumulation of $1.25(OH)_2D_3$ in intestinal mucosa cells (fig.1) and in kidney cells (fig.2). In both tissues the reduced levels of $1,25(OH)_2D_3$ in the cells which were obtained from the cortisol treated rats was found to be accom-

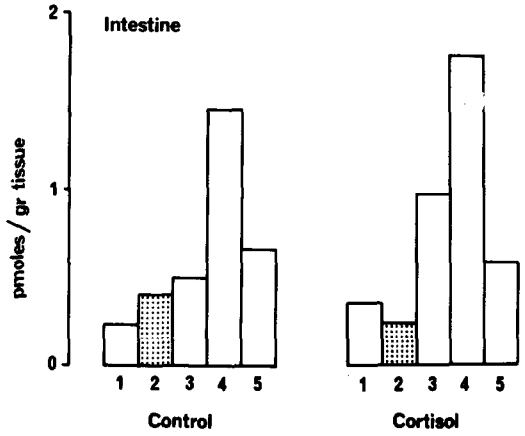

Fig.1. The distribution of cholecalciferol and its metabolites in intestinal mucosa cells of rats treated with cortisol. For experimental details, see sections 2.1 and 2.2. (1.) Polar metabolites; (2) $1,25(OH)_2D_3$; (3) $24,25(OH)_2D_3$; (4) $25(OH)D_3$; (5) Vitamin D_3.

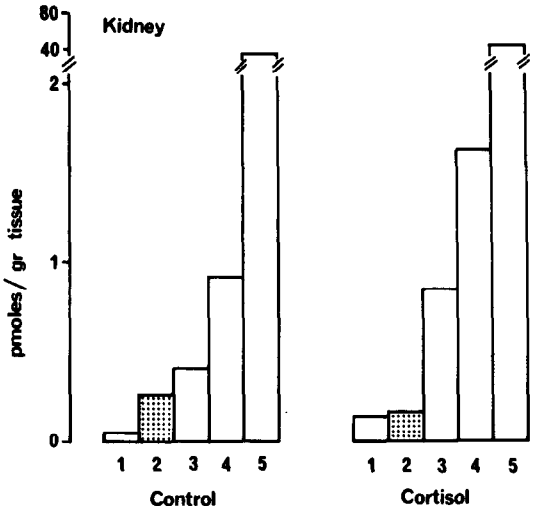

Fig.2. The distribution of cholecalciferol and its metabolites in kidney cells of rats treated with cortisol. For experimental details, see sections 2.1 and 2.2. (1) Polar metabolites; (2) $1,25(OH)_2D_3$; (3) $24,25(OH)_2D_3$; (4) $25(OH)D_3$. (5) Vitamin D_3 and vitamin D_3-esters.

Table 1
The effect of cortisol treatment on the concentration of intestinal CaBP

Treatment	CaBP (^{45}Ca cpm bound/g tissue)	% Control
Propylene-glycol (control)	32.204	100
Cortisol	25.450	79

For experimental details see section 2.3

panied by increased accumulation of 24, 25 $(OH)_2D_3$, a metabolite the production of which by the kidney is known to be inversely related to the production of $1,25(OH)_2D_3$ [15]. As shown in table 1, the reduced levels of $1,25(OH)_2D_3$ in the intestinal mucosa cells obtained from rats treated with cortisol, was associated with a noticeable fall in the concentration of CaBP.

4. Discussion

The presented data on the effect of cortisol treatment on vitamin D metabolism and action, provides for the first time a biochemical basis upon which the well known hypocalcemic effect of glucocorticoids can be explained.

As shown, chronic administration of cortisol to rats, similarly to the manner in which this steroid is used clinically, leads to a decrease in the concentration of $1,25(OH)_2D_3$ in intestinal mucosa cells and kidney cells, and in the intestine this decrease was found to be associated with reduced levels of CaBP. Since the $1,25(OH)_2D_3$ dependent CaBP of both intestine and kidney is believed to be involved in the process of calcium absorption and calcium reabsorption, respectively, the reduced levels of this protein as a consequence of the reduced accumulation of $1,25(OH)_2D_3$ due to cortisol treatment, will result in a net hypocalcemic effect in the intact animal. The mechanism however, by which glucorticoids affect the accumulation of $1,25(OH)_2D_3$ in the target cells, still remains to be established. It is proposed that glucocorticoids in chronic administration, compete with $1,25(OH)_2D_3$ on the receptor sites in the target cells, thus displacing and preventing the interaction of $1,25(OH)_2D_3$ with its functional binding sites. The possibility that the decreased accumulation of $1,25(OH)_2D_3$ in the target cells in response to cortisol treatment is due to decreased production of $1,25(OH)_2D_3$ was ruled out. Spanos et al. [16] have shown recently that chronic administration of cortisol to chicks leads to a profound stimulating effect on the activity of the renal 1-hydroxylase, when measured in vitro.

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