EFFECT OF GLUCOCORTICOIDS ON VITAMIN D METABOLISM

E. SPANOS, K. W. COLSTON and I. MACINTYRE
Endocrine Unit, Royal Postgraduate Medical School, Ducane Road, London W12 OHS, England

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1. Introduction

Vitamin D_3 is hydroxylated in the liver and other tissues to 25-hydroxycholecalciferol ((25-OH)D_3) [1] and further converted by the kidney to 1,25-dihydroxycholecalciferol ((1,25-OH)$_2D_3$) the hormonal form of vitamin D_3 [2].

Evidence has been presented to show that the calcium and phosphorus content of the diet [3,4] 1,25(OH)$_2D_3$ itself [5], parathyroid hormone [6] and prolactin [7,8] regulate the production of (1,25-OH)$_2D_3$ by the kidney. Recently Kenny [9] suggested that steroid hormones stimulate the 25-hydroxycholecalciferol-1α-hydroxylase, the enzyme responsible for the production of (1,25-OH)$_2D_3$ and Tanaka et al. [10] reported that large doses of oestrogens in the presence of androgens have a profound stimulatory effect on the activity of this enzyme. Our results are slightly different since we found that the presence of androgens is not essential for oestrogens to exert their effect. Thus oestrogens alone, or when combined with androgens or even with progesterone, markedly stimulate 1α-hydroxylase activity [11].

The purpose of this report is to present evidence that glucocorticoids also have a profound stimulatory effect on the activity of 1α-hydroxylase.

2. Materials and methods

One-day-old cockerels, Light Sussex–Rhode Island cross-breed were raised on a vitamin D-deficient diet containing 0.34% calcium and 0.54% phosphate. Vitamin D_3 supplements (5 or 20 IU daily injected into the crop in 0.1 ml arachis oil) were given to some chicks beginning at one-day after hatching.

Cortisol was dissolved in propylene glycol and corticosterone in ethanol to the required concentration. The steroids were injected subcutaneously either every 12 h throughout the duration of the experiment or as a single dose. The chicks were killed by decapitation at varying times after the last injection and enzyme activity was assayed in kidney homogenates.

Renal tubules were prepared by a slight modification of the method of Burg and Orloff [12]. Protein estimation was performed by the Folin-Lowry method [13]. Preparation of kidney homogenates and assay of the 25-hydroxycholecalciferol-1α-hydroxylase were as previously described [14,15].

3. Results

The effect of chronic administration of cortisol on the 1α-hydroxylase activity is shown in fig.1. Similarly isolated renal tubules, prepared from vitamin D-deficient chicks, responded to physiological cortisol concentration in the medium by increasing the rate of (1,25-OH)$_2D_3$ production (fig.2). Pentagastrin served as a negative control while the lack of an effect of oestrogens under these conditions was probably due to a very short preincubation time.

When vitamin D-supplemented chicks were used, a single injection of cortisol resulted in the disappearance of 24-hydroxylase activity and in a 20-fold stimulation of the 1α-hydroxylase activity (fig.3).

An early response to steroid hormone treatment was seen using a moderate dose of corticosterone, the chief glucocorticoid in chicks [16] (fig.4).

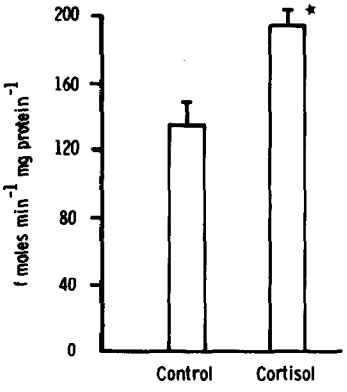

Fig.1. Effect of cortisol on 1α-hydroxylase activity from vitamin D-deficient chicks. 8-Day-old chicks, on a vitamin D-deficient diet, were treated with 500 μg of cortisol every 12 h for 7 days. Kidney homogenates were prepared 18 h after the last injection. Incubation for 10 min at 37°C was carried out in the presence of 12 ng of tritiated (25-OH)D_3.

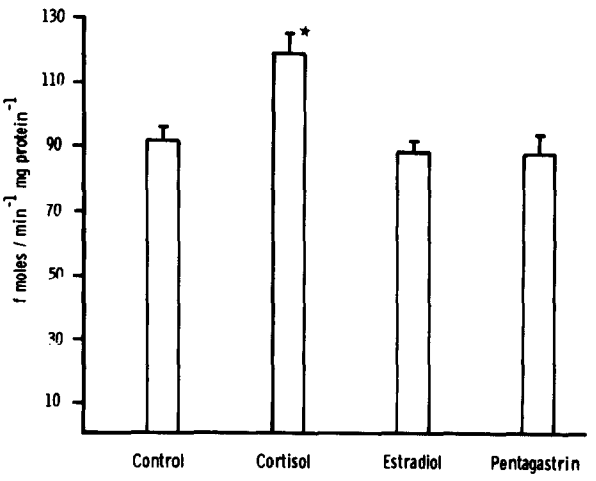

Fig.2. Effect of cortisol on 1α-hydroxylase activity in vitro. Renal tubules were prepared from 24-day-old D-deficient chicks. The renal cells were preincubated for 4 h in the presence of hormones. This was followed by a 10 min incubation in the presence of 12 ng of tritiated (25-OH)D_3. Hormone concentrations were as follows: cortisol 50 ng/ml, β-oestradiol 60 ng/ml and pentagastrin 40 ng/ml.

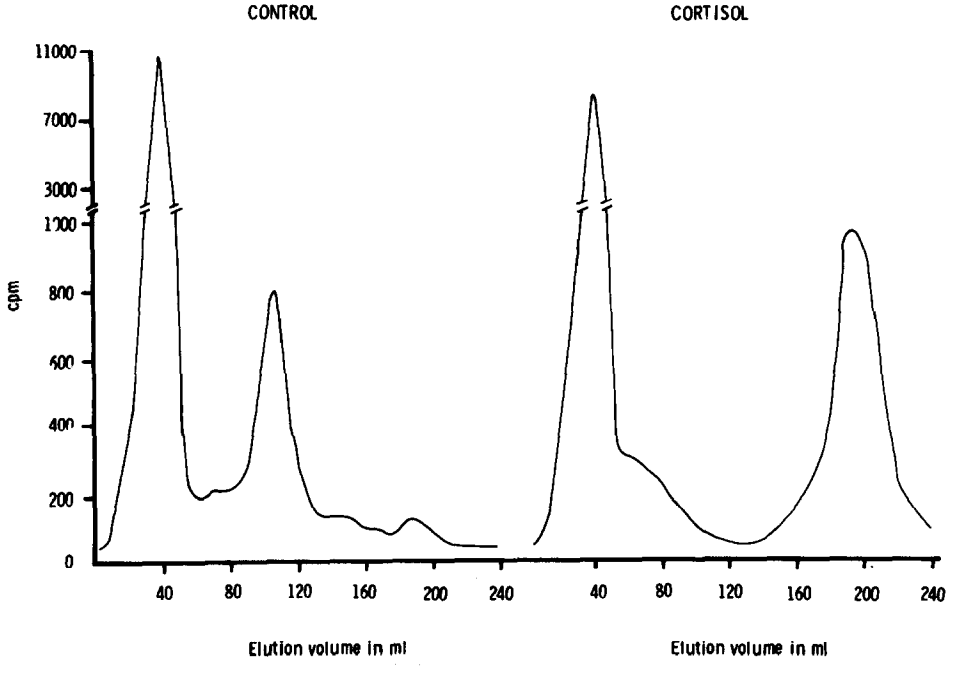

Fig.3. Stimulation of renal 1α-hydroxylase and suppression of 24-hydroxylase by cortisol. A single injection of 2 mg of cortisol was given to 15-day-old chicks, supplemented with 20 IU vitamin D daily. The chicks were killed 16 h later. Kidney homogenates were prepared and incubated for 12 min in the presence of 125 ng tritiated (25-OH)D_3. The lipid extracts were chromatographed on a 12 g Sephadex LH-20 column. Typical elution profiles are shown in fig.3a for controls and fig.3b for cortisol treated animals.

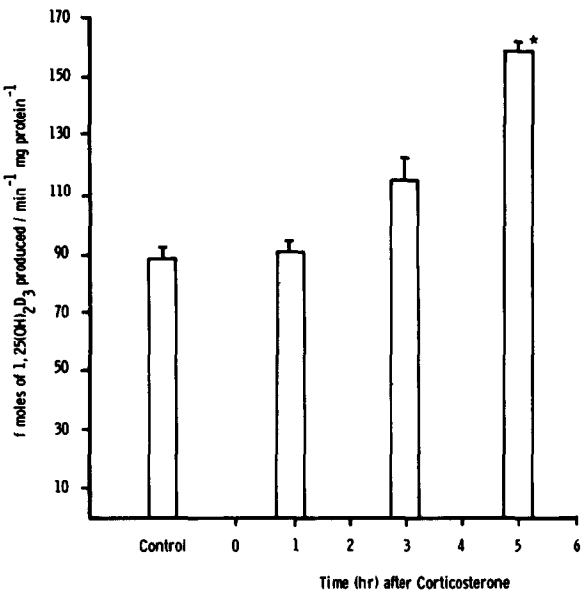

Fig.4. Effect of corticosterone on 1α-hydroxylase activity. 8-Day-old chicks, supplemented with 5 IU vitamin D daily, were given a single injection of 70 μg of corticosterone at 45 min, 3 h and 5 h before sacrifice. Incubation for 10 min was carried out in the presence of 12 ng of tritiated $(25\text{-OH})D_3$.

4. Discussion

The present results clearly show that glucocorticoids stimulate the 1α-hydroxylase activity both in vitamin D-deficient and vitamin D-supplemented chicks either in vivo or in vitro. The differing degree of stimulation observed between these two groups of birds is explained by the fact that in the vitamin D-deficient chicks this enzyme is already stimulated by the lack of vitamin D in the diet [17].

This evidence seems to be in conflict with the well documented inhibitory effect of glucocorticoids on intestinal calcium absorption [18,19]. However, this inhibitory effect is only seen with pharmacological doses of steroids and takes days to develop [20], while the stimulation of the 1α-hydroxylase activity is demonstrated with moderate doses of glucocorticoids within a matter of hours.

Since the effect of glucocorticoids on intestinal calcium absorption is opposite to that of vitamin D, it has been suggested that glucocorticoids may act by antagonizing the action of vitamin D [18,12], or by interfering with the metabolism of vitamin D [21,22]. Avioli and his colleagues [21] reported in 1968 that prednisone administration in man resulted in diminished production of 'active metabolites' (peak IV) and increased production of 'inactive metabolites' (peak V). Since the presumed inactive metabolite in peak V was subsequently identified as $(1,25\text{-OH})_2D_3$ these results seem to be in excellent agreement with our observations.

Kimberg et al. and Favus et al. found that glucocorticoids do not interfere with the metabolism of vitamin D or with the cellular and subcellular localization of $(1,25\text{-OH})_2D_3$ in the intestinal target tissue [20,23,24]. The inability of these investigators to show a stimulatory effect of glucocorticoids on the conversion rate of $(25\text{-OH})D_3$ to $(1,25\text{-OH})_2D_3$ is possibly due to severely D-deficient animals used in their studies. This explanation is supported by our findings as well as by their own observations in vitamin D-supplemented animals, in which the levels of CaBP and bioassayable vitamin D activity in intestinal mucosa from cortisone-treated animals were higher than control animals [20]. A number of studies in vitamin D-supplemented or moderately vitamin D-deficient animals indicates that glucocorticoids may cause an enhanced production of $(1,25\text{-OH})_2D_3$ despite the observed inhibition of intestinal calcium absorption [25–28]. These observations are entirely consistent with, and are explained by, our own findings.

This report presents evidence which rules out the suggestion that the inhibition of intestinal calcium transport by glucocorticoids is due to interference with vitamin D metabolism. Futhermore, these results clearly demonstrate that glucocorticoids stimulate 1α-hydroxylase activity before any inhibition of intestinal calcium transport is observed. The physiological significance of enhanced production of $(1,25\text{-OH})_2D_3$ before and possibly during inhibition of intestinal calcium transport by glucocorticoids remains to be established.

Acknowledgements

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