Effects of Prolactin on DNA Synthesis, Cholesterol Stores, and Steroidogenesis in the Adrenal Cortex of Dexamethasone-Treated Guinea Pigs

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The effect of prolactin on the guinea pig adrenal cortex is studied in a model of dexamethasone-induced atrophy. Prolactin elevates the content of free and total cholesterol per tissue weight but does not affect the specific activity of hydrocortisone synthesized in the presence of labeled cholesterol. It is concluded that prolactin has no direct effect on hormonopoiesis but activates proliferative processes in the adrenal cortex.

Key Words: *prolactin; adrenal cortex; dexamethasone; steroidogenesis; cholesterol*

The adrenal cortex is the target for prolactin. High-affinity prolactin receptors have been described in human and animal adrenals [3,6]. High level of the prolactin-receptor gene expression in adrenocorticocytes has been demonstrated [9]. The pathways of the of prolactin signal transduction in the adrenal cortex have been extensively investigated [11-13].

However, the physiological role of prolactin in the regulation of the adrenal cortex function is unclear. Experiments with guinea pig adrenocortical cells showed that prolactin stimulates cortisol and androgen secretion both in the presence and absence of adrenocorticotropic hormone (ACTH) [8]. We have previously demonstrated that the hormone by itself has no effect on the production of major corticosteroids in intact guinea pigs. On the other hand, prolactin modulates some fundamental biochemical processes. It promotes ^{3}H-thymidine incorporation into DNA, stimulates production, and/or inhibits degradation of the major membrane phospholipid phosphatidylcholine [2]. When administered in combination with ACTH, prolactin considerably potentates its steroidogenic effect without affecting the DNA synthesis. We have hypothesized that prolactin acts as a pleiotropic rather than specific regulator of steroidogenesis in the adrenals, and its role is similar to that of growth factors/cytokines.

The aim of the present study was to assess the influence of prolactin on the adrenal cortex under conditions of atrophy induced by long-term treatment with dexamethasone (DM). The effects of prolactin on the mass of adrenal glands, DNA synthesis, cholesterol stores, and steroidogenesis in the adrenal cortex of DM-treated guinea pigs were examined.

MATERIALS AND METHODS

Experiments were carried out on adult male guinea pigs weighing 300-400 g. The animals were kept at room temperature and natural illumination regime (from the beginning of March till the end of April) and fed *ad libitum*. Dexamethasone was given to some guinea pigs for 7 days in a daily dose of 1 mg/kg body weight. Other guinea pigs received DM and subcutaneous injections of bovine prolactin (20-25 U/mg; Kaunas Endocrine Plant, Lithuania) in a dose of 2 U/100 g body weight in 0.5 ml normal saline. Control animals were injected with the same volume of normal saline.

After termination of DM treatment, 0.5-mm thick sections of the adrenal cortex were prepared. The sections were washed with cooled Eagle's me-

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TABLE 1. Effect of Prolactin on DNA Content and ^{3}H-Thymidine Incorporation into Guinea Pig Adrenal Cortex (*M±m*)

Parameter	Control	DM	DM+prolactin
Mass of adrenals, mg	217.9±29.9 (7)	149.2±21.5* (6)	184.6±22.7 (7)
Mass of adrenals/body weight, mg/g	0.548±0.06 (7)	0.434±0.03* (6)	0.482±0.03 (7)
DNA content, μg:			
per 100 g wet tissue	95.2±17.3 (7)	231.4±33.2** (7)	136.2±16.9+ (7)
per adrenal gland	240.3±31.4 (5)	324.3±44.7 (6)	263.1±57.6 (7)
RNA/DNA	13.1±5.8 (5)	4.0±1.5*** (5)	10.0±4.5 (5)
Specific radioactivity of DNA, dpm/mg×10^{-3}	200.4±48.8 (6)	33.5±4.7** (7)	59.7±10.3+ (7)

Note. Here and in Tables 2 and 3: number of experiments is shown in parentheses; *$p<0.05$, **$p<0.01$ (U test); ***$p<0.055$ (t test) compared with the control; +$p<0.05$ compared with DM.

dium, placed in fresh portions of the Eagle's medium containing 10 mM HEPES (pH 7.4) and 2 mg/ml bovine serum albumin and incubated for 60 min in the presence of 5 μCi/ml ^{3}H-thymidine at 37°C with constant agitation. Radioactivity and DNA content were measured as described previously.

Lipids were extracted from some sections by the method [5]. The cortisol content was determined by radioimmunoassay. The contents of cholesterol and cholesterol esters were measured after fractionation of lipids by thin-layer chromatography [1].

Adrenocorticocytes were isolated and purified as described elsewhere [4], suspended ($0.5\text{-}1.0\times10^6$ cells per sample) in the Eagle's medium supplemented with 10 mM HEPES and 5 mg/ml bovine serum albumin, and incubated at 37°C with constant shacking for 30 min in the presence of 25 μCi/ml ^{3}H-cholesterol. Incubation was stopped by adding a 20-fold volume of chloroform. Chloroform extraction was repeated twice, the extracts were pooled, and evaporated. Corticosteroids were fractionated by two-dimensional thin-layer chromatography, and the radioactivity of newly synthesized hormones was measured and standardized to the mass of the tissue sample.

The data were analyzed using the nonparametric Wilcoxon—Mann—Whitney U and Student t tests.

RESULTS

Treatment with DM for 7 days led to atrophy of the adrenal cortex: the absolute and relative mass of the adrenals decreased (Table 1). The DNA content per tissue weight increased, while the DNA content per adrenal gland remained practically unchanged. This implies that DM treatment leads to shrinkage of adrenocorticocytes without changing their number. A decrease in DNA production was the most obvious manifestation of the DM effect. Specific DNA radioactivity decreased 6-fold (Table 1). The same changes were reported by others [7,14]. A decrease in the RNA/DNA ratio also indicated that proliferative processes were inhibited by DM (Table 1).

Prolactin prevented the DM-induced atrophy: there were no significant differences between the mass of adrenal glands in the control and hormone-treated guinea pigs; the DNA content was close to the control values, while DNA radioactivity increased considerably (Table 1). These findings agree with the observation that prolactin stimulates DNA synthesis

TABLE 2. Effect of Prolactin on the Content and Radioactivity of Cholesterol and Cholesterol Esters in the Guinea Pig Adrenal Cortex (*M±m*)

Parameter	Control	DM	DM+prolactin
Free cholesterol			
content, μg/mg tissue	8.14±1.02 (7)	8.69±0.99 (7)	12.71±1.80+ (7)
radioactivity, dpm/mg tissue	83±13 (6)	112±10 (5)	166±25+ (7)
Cholesterol esters			
content, μg/mg tissue	72.1±14.6 (7)	69.6±10.4 (7)	85.1±15.8 (7)
radioactivity, dpm/100 mg tissue	277±72 (6)	566±163 (6)	471±134 (7)
Total cholesterol			
content, μg/mg tissue	80.2±15.2 (7)	78.3±11.3 (7)	107.7±14.1+ (6)
free/esterified	0.137±0.02 (7)	0.133±0.013 (7)	0.196±0.050 (7)

TABLE 3. Effect of Prolactin on ^{3}H-Cholesterol Incorporation into Corticosteroids by Guinea Pig Adrenocorticocyte Suspension (*M±m*)

Corticosteroid	Control	DM	DM+prolactin
Cortisol, dpm/100 mg tissue ($\times 10^{-3}$)	4.01±1.44 (7)	8.64±2.37* (6)	4.15±0.78* (6)
dpm/μmol cortisol	498±137 (7)	536±96 (5)	535±175 (7)
Cortisone, dpm/100 mg tissue ($\times 10^{-3}$)	4.51±0.50 (6)	7.73±1.85** (5)	9.51±2.12 (7)
Corticosterone, dpm/100 mg tissue ($\times 10^{-3}$)	3.61±0.66 (7)	8.19±1.94 (6)	5.86±0.91 (7)
Aldosterone, dpm/100 mg tissue ($\times 10^{-3}$)	3.48±0.65 (7)	5.92±0.74* (6)	3.57±0.76* (6)

in guinea pig adrenal cortex and show that the hormone activates proliferative processes in the adrenals against the background of atrophy.

Dexamethasone practically did not change the cholesterol stores in the adrenal cortex. There were no significant changes in the content and radioactivity of cholesterol and cholesterol esters after incubation of isolated adrenocorticocytes with ^{3}H-cholesterol (Table 2). Prolactin induced a statistically significant rise of free and total cholesterol in the adrenal cortex and increased the radioactivity of free cholesterol. Thus, it can be hypothesized that prolactin stimulates cholesterol accumulation in the adrenal glands but does not participate in activation or inhibition of steroidogenesis. Our findings are insufficient for elucidation of the mechanism underlying the effect of prolactin on the cholesterol content and radioactivity. However, prolactin stimulates the binding of low density lipoproteins, the major source of cholesterol in steroidogenic cells, to prolactin-sensitive luteocytes [10].

Dexamethasone stimulated the incorporation of ^{3}H-cholesterol into all measured corticosteroids (Table 3) and did not change specific radioactivity of cortisol, the major glucocorticoid in the guinea pig. The DM-induced increase in the corticosteroid radioactivity was probably due to atrophy and, consequently, to cell shrinkage (Table 1), which elevates radioactivity per tissue weight. Since prolactin prevents the DM-induced shrinkage, it abolished the effect of DM on the radioactivity of steroids (Table 3). Presumably, prolactin does not influence steroidogenesis, which agrees with our previous findings.

Thus, prolactin stimulates proliferative processes in the adrenal cortex under conditions of DM-induced atrophy mediated by inhibition of corticotropin secretion in the pituitary. Prolactin may act by stimulating the DNA synthesis and cell division, which is accompanied by an increase in cholesterol stores. This mechanism presumably maintains adequate secretion of corticosteroids by preserving the mass of the adrenals.

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