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INHIBITION OF LYSYL OXIDASE AND PROLYL HYDROXYLASE ACTIVITY IN GLUCORCORTICOID TREATED RATS

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SUMMARY

Triamcinolone diacetate produced a dose dependent decrease in lysyl oxidase activity in the skin of new born rats when administered over a three day period. Maximum inhibition by this glucorticoid resulted in less than 10% of control lysyl oxidase activity. A similar though less dramatic effect was observed on skin prolyl hydroxylase activity. These results suggest that the antianabolic effect of glucocorticoids on collagen synthesis extend to enzymes involved in the intra- and extracellular modifications of collagen.

Glucocorticoids have a significant inhibitory effect on growth in humans (1) and experimental animals (2,3). DNA synthesis (3), protein synthesis in general (3,4,5) and collagen synthesis in particular (3,5,6) are decreased in skin following glucocorticoid treatment. In newborn rat skin the selective decrease in collagen synthesis is accompanied by coordinate reductions in the intracellular enzymes prolyl hydroxylase (E.C. 1.14.11.2) and lysyl hydroxylase (E.C. 1.14.11.4) (3). Activity of collagen galactosyltransferase (E.C. 2.4.1.50) and glucosyltransferase (E.C. 2.4.1.66) is also inhibited in cortisol treated chick embryo cells (7). The hydroxylation of prolyl residues is necessary for efficient collagen secretion and helix stability whereas hydroxylysine residues are sites for collagen glycosylation and crosslink formation (8,9). The formation of

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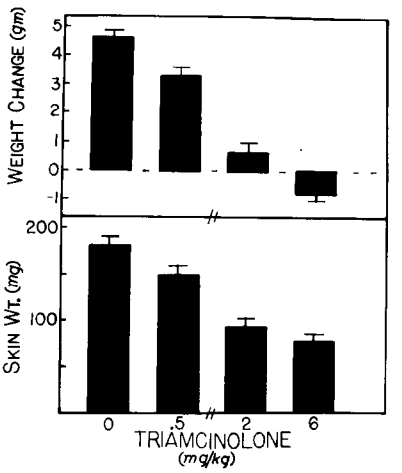

Figure 1:

Skin and body weight changes following triamcinolone diacetate treatment. One day old rats received three daily injections (0.1 ml) of triamcinolone diacetate at the indicated concentration. Skins were removed, cleaned of fascia and musculature, blotted dry and weighed. Values represent means ± S.E. of determinations from four to six animals.

inter- and intramolecular crosslinks is an important extracellular modification of collagen. Crosslinking is initiated by the copper-dependent enzyme lysyl oxidase (10,11). In this reaction the ε-amino groups of certain lysyl and hydroxylysyl residues are oxidatively deaminated to form the corresponding ε-semi-aldehydes. These then condense either with ε-amino groups of other lysyl or hydroxylysyl residues or with other semi-aldehydes to form Schiff base or aldol crosslinks (11,12). Decreases in lysyl oxidase by administration of lathrogens (10,13,14), copper deficiency (15), pyridoxal deficiency (16) or hypophysectomy (17) can result in decreased crosslinking and increased solubility of collagen (10,13,17).

No data is available on the effect of glucocorticoids on lysyl oxidase activity. The present report confirms the inhibitory effect of triamcinolone diacetate on prolyl hydroxylase activity and further demonstrates that this synthetic glucocorticoid dramatically decreases skin lysyl oxidase in a dose dependent manner.

MATERIALS AND METHODS

Wistar rats (1 day old) were injected intraperitoneally daily for three days with powdered triamcinolone diacetate (Sigma) suspended in 0.9% (w/v) NaCl. Animals were killed 8 hours after the last injection by decapitation, their skins removed, cleaned of fascia and musculature and blotted dry. Skins were rinsed in Tris buffered saline (TBS: 0.05 M Tris-HCl, 0.15 M NaCl, pH 7.5) and homogenized in 4 volumes of TBS with a Polytron ST-10 for 30 sec. at maximum speed. The homogenate was centrifuged at 20,000g for 20 min. and the supernatant dialyzed against TBS for twelve hours and assayed for prolyl hydroxylase activity. The 20,000g pellet was resuspended in 4 volumes TBS, 6M urea, homogenized and centrifuged as described above. The supernatant was removed, dialyzed overnight against TBS and assayed for lysyl oxidase activity. Prolyl hydroxylase activity was assayed by the tritium release method of Peterkofsky and DiBlasio (18) using chick embryo calvaria [4-^{3}H]-proline protocollagen substrate. Lysyl oxidase activity was also assayed by tritium release using chick embryo [4,5-^{3}H]-lysine labeled calvaria collagen by the method of Melet et al. (19) employing a 3 hour incubation period. All experiments included enzyme-free controls and lysyl oxidase activity was 95% inhibited by adding β-aminopropionitrile to the assay at 50 μg/ml. Protein was determined by the method of Bradford (20) using bovine serum albumin as standard. [4-^{3}H]-proline (24 Ci/mmole) and [4,5-^{3}H] lysine (55 Ci/mmole) were purchased from ICN (Irvine, CA).

RESULTS AND DISCUSSION

Injections of triamcinolone diacetate resulted in a dose-dependent decrease in skin weight and a significant inhibition in body weight gain over a three day period (Figure 1). Animals receiving 6mg/kg actually lost body weight during the treatment. This effect is most likely due to the well documented catabolic effects of glucocorticoids in skin, musculature and other peripheral tissues (2, 21-24).

Skin lysyl oxidase and prolyl hydroxylase specific activity decreased significantly in a dose dependent manner (Table 1). The 51% inhibition of prolyl hydroxylase activity is similar to the maximum inhibition others have observed in newborn rat skin at higher doses of glucocorticoids (3). The reason for the greater inhibitory effect of triamcinolone diacetate on lysyl oxidase in unknown but may reflect a shorter half life for lysyl oxidase compared to prolyl hydroxylase. Chick lysyl oxidase has a half-life of approximately 16 hours (25) whereas chick prolyl hydroxylase has been estimated at 40 hours (26). Similar data is not available for newborn rat skin but we are presently examining this point. Triamcinolone diacetate had no effect on either enzyme activity when added directly to

Table 1 Dose-response of skin lysyl oxidase and prolyl hydroxylase activity to triamcinolone diacetate

Triamcinolone dose (mg/kg)	Lysyl oxidase activity cpm x 10^{-3}/mg protein	Prolyl hydroxylase activity cpm x 10^{-3}/mg protein
0	9.2 ± .92	36.1 ± 1.5
0.5	7.2 ± .84 (22%)[a]	29.2 ± 1.5 (19%)[a]
2.0	4.1 ± 1.6 (55%)[b]	21.1 ± .96 (42%)[b]
6.0	.83 ± .30 (91%)[b]	17.6 ± .50 (51%)[b]

One day old rats were injected intraperitoneally for 3 consecutive days with triamcinolone diacetate at the doses indicated and were sacrificed 8 hours after the last injection. Lysyl oxidase was assayed as described in the text and is expressed as the quantity of [^{3}H] water formed after 3 hours from [4,5-^{3}H]-lysine labeled chick embryo collagen substrate. Prolyl hydroxylase is expressed as the quantity of [^{3}H] water formed after 20 min from [4-^{3}H]-proline chick embryo protocollagen substrate. The values represent the means ± S.E. of enzyme activity from four to six animals. The numbers in parentheses are the percentage decrease of enzyme activity compared to controls.

[a]Significantly different from control at $p \leq .05$

[b]Significantly different from control at $p \leq .01$

the reaction mixture at 100 µg/ml (results not shown). Other investigations have noted the glucocorticoid induced decrease in prolyl hydroxylase acitivity but our results extend the antianabolic effect of glucocorticoids to the extracellular enzyme lysyl oxidase. This result is consistent with other investigations noting coordinate changes in collagen synthesis and collagen processing enzymes in other tissues during periods of rapid cell proliferation and fibrosis (27-31).

The relationship of decreased lysyl oxidase activity to glucocorticoid induced alterations in skin collagen metabolism is unclear at this time. Although conflicting reports exist (32-34), it has been suggested that glucocorticoids may enhance collagen degradation (24, 35,36) and glucocorticoids have been reported to increase skin collagenolytic and proteolytic activity (22,23). In this regard inhibition of lysyl oxidase will result in the production of uncrosslinked collagen which is more susceptible to degradation by tissue collagenases (10,38,39).

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