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Temporal response of prolyl hydroxylase in pre-existing granuloma to glucocorticoid administration*

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Administration of triamcinolone diacetate, a synthetic fluorinated glucocorticoid, results in a decrease of prolyl hydroxylase, the enzyme catalysing the hydroxylation of certain prolyl residues in collagen to form hydroxyproline, in liver, lung, heart, aorta and skin (Cutroneo et al 1975; Newman & Cutroneo 1978; Oikarinen 1977). However, reports from other laboratories have indicated that prolyl hydroxylase activity is not altered in both bone (Uitto & Mustakallio 1971) and in pre-existing granuloma tissue (Nakagawa et al 1971; Nakagawa & Tsurufuji 1972) following glucocorticoid treatment. We have determined the temporal response of prolyl hydroxylase to steroid treatment in pre-existing granuloma tissue, liver and skin tissues. Our data indicate that enzyme activity decreases in skin and granuloma tissues following multiple daily injections of drug. After a single injection of drug, only the liver enzyme activity was decreased.

Intact 90–110 g male albino Sprague-Dawley rats were supplied by Carsworth Farms, Rockland County, N.Y. All chemicals were analytical reagent grade. Commercial corticoid preparations were: Hydrocortisone acetate (Hydrocortone; Merck, Sharp and Dohme, West Point, Pa.); betamethasone maleate (Schering Corp., Bloomfield, N.J.). Powdered triamcinolone diacetate was supplied by Dr E. W. Cantrell of Lederle Laboratories, Pearl River, N.Y. Saline suspensions of the corticoids were prepared by adding the steroid to 0·9% (w/v) NaCl (saline) at a concentration of 5 mg ml^{-1} and mixing with a Potter-Elvehjem homogenizer fitted with a Teflon-coated pestle to a smooth suspension.

Enzyme activities in the 15 000 *g* supernatant from homogenates of liver, skin and granuloma tissues were measured by a modification (Cutroneo et al 1975) of the tritium release assay of Hutton et al (1966) using chick embryo substrate. Tritium release was linearly related to the time of incubation and was proportional to the amount of enzyme. Final radioactivity in the experimental samples was 4 to 5 times the radioactivity of the blank prepared by incubating the substrate without enzyme. All enzyme assays were carried out on at least two levels of 15 000 *g* supernatant to ensure linearity in relation to enzyme concentration. Enzyme activities can only be compared within a Table or figure since different substrates having different activities for the same enzyme preparation were used throughout these studies. Protein concentration in the 15 000 *g* supernatant was determined (Lowry et al 1951) using bovine serum albumin (Calbiochem, Los Angeles, Calif.) as standard.

Sub-dermal implants of polyvinyl sponges were used to induce granuloma tissue growth. The sponges (10 mm diam. 42 mm length, Scientific Products) were washed in running water for 48 h, sterilized for 15 min and dried. Before implantation they were rinsed in sterile saline. The animals were anaesthetized with ether. A trocar was inserted and guided to the dorsothoracic region and the sponge implanted. On the fifth day after sponge implantation, the animals were administered 50 mg kg^{-1} i.p. of the corticoid suspension (a dose of 20 mg kg^{-1} had not decreased prolyl hydroxylase activity in carregeenan-induced granuloma tissue, Nakagawa et al 1971, nor was any decrease observed in chick embryo tibiae of birds receiving a dose of approximately 100 mg kg^{-1} (Uitto & Mustakallio 1971). Controls were treated with saline. The animals were killed by cervical dislocation 12 h after a single injection or 24 h after the last of several injections. The granuloma tissue was removed from the sponge and weighed frozen in liquid nitrogen and pulverized. A 10% (w/v) homogenate in 0·25 M sucrose, 10^{-5} M ethelenediamine tetracetic acid, 10^{-5} M dithiotheritol, and 0·05 M Tris-HCl (pH 7·5) was prepared using a Polytron ST-10 system (Kinematica GmbH, Luzern, Switzerland). The homogenates were centrifuged for 15 min at 4 °C in an International B-60 centrifuge at 15 000 *g* and the supernatant used as the enzyme source. All animals were killed at 9 a.m. to avoid diurnal variation (Cutroneo & Scott 1973). A 2·5 cm square area of the ventral abdominal skin was

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Table 1. Glucocorticoid-induced decrease of prolyl hydroxylase activity in pre-existing granuloma tissue. Granuloma was allowed to form for 5 days. The animals were then treated with the drug indicated (50 mg kg^{-1} i.p.) for 5 days. The animals were then killed and the tissues assayed as described in the text. Enzyme activity was expressed as the quantity of [^{3}H]water formed in 30 min mg^{-1} of 15 000 *g* supernatant protein added to the assay mixture. The values represent the mean ± s.e. of data from 6 animals. * Significantly different from control at $P \leq 0.05$.

Treatment	Prolyl hydroxylase activity d. min^{-1} [^{3}H]water formed $\times 10^{-3}$ mg^{-1} protein)
Control	5·5 ± 0·4
Trimacinolone	2·5 ± 0·2*
Betamethasone	1·9 ± 0·2*
Hydrocortisone	2·4 ± 0·3*

Table 2. Dose dependent decrease of granuloma prolyl hydroxylase activity by triamcinolone diacetate. Granulomas were implanted and animals were treated with various doses of triamcinolone deacetate as described in the lengend of Table 1. The values represent the mean ± s.e. of data from 6 animals. * Significantly different from control at $P < 0.05$.

Dose (mg kg^{-1})	Prolyl hydroxylase activity d. min^{-1} [^{3}H]-water formed $\times 10^{-3}$ mg^{-1} protein	Inhibition %
Control	14·4 ± 0·8	
0·7	12·0 ± 0·8*	17
2·0	10·6 ± 1·3*	27
7·0	6·3 ± 0·7*	57
20·0	4·4 ± 0·9*	69

shaved, dissected free, frozen in liquid nitrogen and pulverized. A 10% (w/v) tissue homogenate was prepared as described for granuloma tissue. The liver was rinsed in saline and homogenized. The 15 000 *g* supernatant fractions of skin and liver homogenates were prepared for enzyme assay and protein determination.

Table 1 shows the effect of the administration of various corticoids on prolyl hydroxylase activity in pre-existing granuloma tissue. Decreased prolyl hydroxylase activity is observed in granuloma tissue in animals treated with either synthetic or naturally occurring glucorcorticoids.

The response of decreased enzyme activity to glucocorticoid administration is also dose-dependent (Table 2). Administration of various doses of triamcinolone diacetate ranging from 0·7 to 20 mg kg^{-1} i.p. results in a dose-dependent decline of enzyme activity in granuloma tissue.

Nakagawa et al (1971) reported that there was no decrease in granuloma prolyl hydroxylase activity after a single injection of betamethasone. To determine when the decrease in prolyl hydroxylase occurred, groups of animals bearing granuloma were treated daily with triamcinolone diacetate and killed at various times following the initial injection. As shown in Fig. 1, 12 h after the initial injection of glucocorticoid, there was a significant decrease of prolyl hydroxylase activity only in the liver. Forty-eight hours after the initiation of drug treatment significant decreases of prolyl hydroxylase were also observed in granuloma and skin tissues. The differences in the temporal response of prolyl hydroxylase in different tissues may be a function of the route of drug administration.

The temporal decrease of prolyl hydroxylase was observed in granuloma, skin and liver tissues after intraperitoneal injection of glucocorticoid to rats. The data indicate that the decrease in prolyl hydroxylase activity requires more than 12 h for expression in skin and granuloma tissue, but not in liver tissue. Thus, when determining the relationship of the decrease in

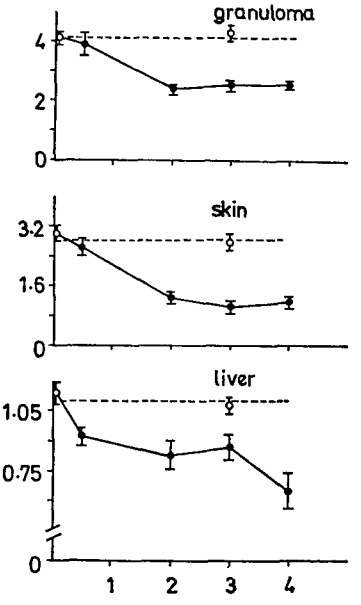

FIG. 1. Temporal response of prolyl hydroxylase in granuloma, skin and liver following glucocorticoid administration. Weanling rats with granulomas were treated with triamcinolone as described. Animals were killed after treatment with triamcinolone on the days indicated. Control animals were killed at 0 day and the 3rd day after initiation of drug treatment. A statistically significant difference was not observed between 0 day and 3 day control animals. The dotted line represents the average prolyl hydroxylase value for control animals. Prolyl hydroxylase activity was determined as described in the text and was expressed as the quantity of [^{3}H] water formed in 30 min per mg of 15 000 × *g* supernatant protein added to the assay mixture. Values represent the mean ± s.e. data from 3–6 animals. Ordinate: prolyl hydroxylase activity (d min^{-1}[^{3}H] water formed $\times 10^{-3}$/mg protein). Abscissa: days of treatment.

prolyl hydroxylase to other processes involved in collagen biosynthesis such as synthesis, degradation and cellular secretion of collagen, certain tissues from animals treated for multiple days must be utilized.

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Metergoline antagonism of 5-hydroxytryptamine-induced activation of rat cerebral cortical (Na^+-K^+)ATPase

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It has been known for some time that biogenic amines (namely, noradrenaline, 5-hydroxytryptamine (5-HT), dopamine and histamine) produce a depressant effect on cerebral cortical and other central neurons (Krnjevic & Phillis 1963; Phillis 1970; Yarbrough et al 1974). The studies investigating the underlying mechanisms of biogenic amine action on neurons have suggested that amines may inhibit neurons by stimulating an electrogenic ion pump (Phillis 1976; Phillis et al 1978). Sastry & Phillis (1977) have shown that biogenic amine-induced depression of cerebral cortical neurons can be antagonized by (Na^+-K^+)-ATPase inhibitors and suggested that a major component of depressant action of monoamines may result from stimulation of an electrogenic (Na^+-K^+)-ATPase, an enzyme known to be associated with sodium pumping.

The biogenic amine-induced activation of (Na^+-K^+)-ATPase has been well documented (Schaefer et al 1972; Yoshimura 1973; Godfraind et al 1974; Lee & Phillis 1977; Phillis et al 1978). The mechanism by which noradrenaline exerts its effect on the (Na^+-K^+)-ATPase has also been examined. Noradrenaline stimulation o-(Na^+-K^+)-ATPase is antagonized by both α- and βt adrenoceptor blockers (Iwangoff et al 1974; Gilberf et al 1975; Wu & Phillis 1978) indicating that α- and β-adrenoceptors might be involved in the noradrenaline stimulation of (Na^+-K^+)-ATPase. The possibilities that 5-HT exerts its action on central neurons by a similar mechanism, namely, the stimulation of (Na^+-K^+)-ATPase, therefore warrant consideration. 5-HT activates brain (Na^+-K^+)-ATPase (Logan & O'Donovan 1976; Lee & Phillis 1977). However, the underlying mechanism(s) by which it does so are still poorly understood.

In the present investigation we have studied the stimulating effect of 5-HT and its structural analogues on the (Na^+-K^+)-ATPase of cerebral cortical homogenates, as well as the possibility of a 5-HT receptor involvement in the enzyme activation.

The cortex from male Sprague Dawley rats (200–300 g) was removed and homogenized in 50 vol of distilled H_2O (pH 7·5 buffered by Tris-HCl solution). Some of this homogenate (50 μl) was used for the incubation. The (Na^+-K^+)-ATPase activity was determined by subtracting Mg^{2+}-ATPase (ouabain insensitive) from total ATPase activity. The medium used for the estimation of total ATPase activity consisted of (mM) in final concentration: Tris, 115; $MgCl_2$, 5·0; KCl, 6·25; NaCl, 72·5; ATP, 2 pH 7·5. Mg^{2+}-ATPase activity was measured in the K^+-free medium which was composed of (mM) in final concentration: Tris, 172·5; $MgCl_2$, 5·0; NaCl, 14; ouabain, 1·0 and ATP, 2 pH 7·5. In all experiments, the homogenate was preincubated for 10 min at 37 °C in the presence of agonist and/or antagonist. The reaction was terminated 6 min after the addition of disodium adenosine triphosphate (ATP) by adding 1·0 ml ice-cold 12% trichloroacetic acid. The content of inorganic phosphate in the supernatant was measured by the method of Fiske & Subbarrow (1925). The reagents were made up freshly for every experiment. Metergoline was dissolved in small amounts of ethanol and subsequently diluted to a final concentration of 10^{-5} M containing 0·08 M ethanol.

Adenosine-5'-triphosphate(ATP) (Grade 1, synthesized by phosphorylation of adenosine), 5-hydroxytryptamine-oxalate, 5-hydroxyindolacetic acid and tryptamine were purchased from Sigma Chemical Co. (Mo., U.S.A.). Metergoline was a gift from Drs. Praga and Ghione of Farmitalia. Other chemicals were of analytical grade.

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