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Effect of ACTH on cytochrome P-450 content and DMBA metabolism in immature rat adrenal

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The aromatic hydrocarbon 7,12-dimethylbenz-[*a*]anthracene (DMBA)* is one of the most potent chemical carcinogens known. It causes cancer in several organs, e.g. the liver, lung, skin, ovary and mammary gland [1]. In rat adrenal cortex and testis DMBA but not BP or other polycyclic aromatic hydrocarbons causes necrosis. The two inner zones of the adrenal cortex are destroyed and this effect is dependent on endogenous factors, e.g. ACTH [1] and estradiol [2]. Protection against necrosis may be obtained by certain cytochrome P-450 inhibitors and inducers [3].

However, the adrenals are unaffected if DMBA is administered to immature or hypophysectomized mature rats, but necrosis is induced if ACTH is co-administered [4]. In mature rats this has been shown to be related to an ACTH-dependent cytochrome P-450 content and BP hydroxylase activity [5]. However, the consequences of ACTH administration to immature rats have not been investigated with regard to changes in enzyme levels involved in detoxification.

Treatment of rats in vivo *with ACTH and DMBA.* Experiments were carried out with juvenile (20 days old, 40 g) and adult (50 days old, 180 g) female Sprague–Dawley rats. Both classes of rats were divided into four groups (control, ACTH, ACTH plus DMBA, and DMBA groups) each containing five rats. Juvenile rats were treated with ACTH for 10 consecutive days prior to decapitation. The ACTH and the ACTH plus DMBA groups were injected intramuscularly with ACTH (8 I.U./day) alone or plus a single intraperitoneal injection of DMBA (25 mg/kg, dissolved in corn oil) 3 days before decapitation. ACTH was dissolved as a lyophilized powder in an acidic saline buffer (pH 2). In the case of the DMBA group only DMBA was administered in this manner. Control and ACTH groups received corn oil (2.8 ml/kg). Treatment of adult rats was carried out in a similar way except that the rats received 4 I.U. of ACTH/day for seven consecutive days. The intraperitoneal dose of DMBA was 50 mg/kg in this case.

Adrenal microsomes from each group of five rats were pooled and prepared as described by Ogle [6]. Determination of DMBA metabolism was carried out with HPLC analysis and by a distribution assay [7]. The content of cytochrome P-450 was estimated according to Omura and Sato [8].

ACTH (Synacten Depot type) was generously donated by Ciba-Geigy (Basel, Switzerland). DMBA and [^{14}C]dimethyl-DMBA were purchased from Sigma Chemical Co. (St. Louis, MO) and NEN (Dreieichenbahn, F.R.G.), respectively.

Effect of ACTH on cytochrome P-450 and AHH. Adrenal microsomes isolated from control immature female Sprague–Dawley rats converted DMBA at a rate of 48.3 pmoles/min/mg protein (Table 1). Administration of ACTH increased this activity almost two-fold. Typically, the content of microsomal cytochrome P-450 showed a similar increase following ACTH administration (Table 1). In absolute terms the values of the control rats were considerably higher than those reported by Jellinck *et al.* [9] who found a complete lack of AHH activity with DMBA as substrate in 10–15-day-old untreated rats. This discrepancy may be due to the fact that 30-day-old rats were used in the present investigation. Table 1 shows the cytochrome P-450 content and AHH activity of adrenal microsomes isolated from adult female Sprague–Dawley rats. In this case the AHH activity was 86.3 pmoles/min/mg protein with DMBA as the substrate; administration of ACTH did not change cytochrome P-450 content or AHH activity significantly.

DMBA administration caused a slight decrease in DMBA metabolism in both ACTH-treated as well as untreated immature or mature rats (Table 1). This lower activity may be due to the presence of unmetabolized DMBA in the microsomal membranes during the assay, derived from injected DMBA, or reflect inactivation of the DMBA-metabolizing enzymes.

Conclusion. The present results show that adrenal microsomal cytochrome P-450 and AHH are lower in immature female rats than in mature ones and that ACTH administration markedly decreases this difference by inducing cytochrome P-450 and AHH in the immature rats. It is thus conceivable that the low and ACTH-dependent levels of

* Abbreviations: AHH, aryl hydrocarbon hydroxylase; DMBA, 7,12-dimethylbenz[*a*]anthracene; BP, benz-[*a*]pyrene; ACTH, adrenocorticotropic hormone.

Table 1. DMBA metabolism and cytochrome P-450 content in microsomes from immature and mature female rat adrenals*

Treatment	Cytochrome P-450 content (nmoles/mg protein)†	DMBA metabolism (pmoles formed/ min/mg protein)‡	P
Immature rats			
Control	0.18	48.3 ± 1.1	—
+ ACTH	0.29	91.8 ± 1.2	< 0.001
+ ACTH/DMBA	0.28	84.9 ± 1.7	< 0.01
+ DMBA	0.19	37.0 ± 0.1	< 0.01
Mature rats			
Control	0.49	86.3 ± 5.4	—
+ ACTH	0.43	97.2 ± 10.6	NS§
+ ACTH/DMBA	0.39	83.4 ± 1.1	NS
+ DMBA	0.45	85.8 ± 5.8	NS

* Rats were treated with ACTH and/or DMBA as indicated. The concentration of DMBA was 50 μM.

† Typical results from single experiments with microsomes from pooled adrenals from five rats as indicated in the text.

‡ Mean ± S.D.; duplicate experiments with microsomes from pooled adrenals from five rats as indicated in the text.

§ No significant difference.

cytochrome P-450 and AHH in these adrenals provide an explanation for the diminished sensitivity to DMBA. The fact that adult rats are unaffected by ACTH in these respects indicates that maturation is associated with a marked enhancement of the capacity of the adrenal to metabolize polycyclic aromatic hydrocarbons and drugs.

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Recovery of prostacyclin production by cultured bovine smooth muscle cells after aspirin inhibition: effect of serum replacement and concentration in culture medium

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Arachidonic acid (AA) is converted to PGI_2 in the cells of the vessel wall. This conversion requires a cyclooxygenase-catalyzed step. Aspirin irreversibly inactivates cyclooxygenase, thus inhibiting PGI_2 generation [1]. After aspirin treatment vascular cells recover their ability to produce PGI_2 by synthesizing new enzyme [2]. There are still wide discrepancies however as regards the time for regeneration of PGI_2 synthesis when cultured vascular cells are used. In cultured endothelial cells from the human umbilical vein it has been reported to take 36 hr by one group [2] and 2 hr to recover 50% by another [3]. Cultured porcine aortic endothelial cells seem to require 24 hr [4] and smooth muscle cells from the rat aorta 1 hr [5]. The serum concn in culture media greatly influences cellular events such as protein synthesis by different cells [6, 7]. The aim of the present study was to assess whether supplementation of fresh serum at different concns influenced the recovery of PGI_2 synthesis in aspirin-treated vascular