Effect of short-term exposure of rats to dehydroepiandrosterone on the hepatic metabolism of dimethylnitrosamine

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The influence of short-term treatment with dehydroepiandrosterone (DHEA), a naturally occurring adrenal steroid, on hepatic metabolism and macromolecular interactions of the hepatocarcinogen dimethylnitrosamine (NDMA) was investigated in male Sprague–Dawley rats. Liver weight, total tissue protein ($P < 0.05$), microsomal and cytosolic proteins and cytochrome *P*-450 ($P < 0.001$) were all significantly increased in rats treated orally with DHEA (300 mg/kg body wt., suspended in 1.0 ml of sesame oil). The hepatic DNA content was not altered, however. Methylation of DNA by NDMA was reduced significantly in DHEA-treated rats ($P < 0.05$). The binding of [¹⁴C]NDMA to hepatic proteins was greater in DHEA-treated rats. The results suggest that short-term treatment of rats with DHEA enhances the binding of NDMA-derived metabolites to hepatic proteins, resulting in the protection of DNA from the damaging effects of NDMA.

INTRODUCTION

Chemoprevention of cancer by several natural and synthetic chemicals has been intensely investigated (Wattenberg, 1985). Dehydroepiandrosterone (DHEA), a naturally occurring adrenal steroid, has been gaining prominence in this area of study by virtue of its ability to inhibit both chemically induced and spontaneously formed tumours (Schwartz *et al.*, 1988). Although DHEA has been documented to inhibit both the initiation and promotion phases of tumourigenesis, a paucity of data exists on its mechanism of action (Gordon *et al.*, 1987).

Previously, we reported that DHEA inhibited the binding of the carcinogen 7,12-dimethylbenz[*a*]anthracene to hepatic DNA (Prasanna *et al.*, 1989), suggesting an influence of the steroid on the metabolic activation of carcinogens. In the present study, we have shortened the duration of DHEA exposure to 48 h in order to determine whether the metabolism of the hepatocarcinogen dimethylnitrosamine (NDMA) (Magee, 1956) is influenced by DHEA.

MATERIALS AND METHODS

DHEA and NADPH were obtained from Sigma, St. Louis, MO, U.S.A.; 5,5-dimethyl-1- β ,3-cyclohexane from Eastman Kodak, Rochester, NY, U.S.A.; [¹⁴C]dimethylamine (specific activity, 54 mCi/mmol) was supplied by New England Nuclear Corp., Boston, MA, U.S.A. and radiolabelled NDMA was prepared by the nitrosation of [¹⁴C]dimethylamine as previously described (Dutton & Heath, 1956). The radiochemical purity of the [¹⁴C]NDMA, as determined by h.p.l.c., was 98%. Unlabelled NDMA was purchased from Aldrich Chemical Co., Milwaukee, WI, U.S.A. All other chemicals used

were of analytical grade and were obtained from Fisher Scientific Co., Fairlawn, NJ, U.S.A.

Treatment of animals

Male Sprague–Dawley rats of approx. 180 g body wt. were purchased from Harlan Sprague–Dawley, Inc., Indianapolis, IN, U.S.A. They were individually housed in metal cages and maintained on Wayne Lab-blox diet for 7–10 days before use. The animals were divided into two groups, controls and DHEA-treated. DHEA was administered by gavage (300 mg/kg body wt., suspended in 1.0 ml of sesame oil). After 24 h, a second dose of DHEA was given. On both of these occasions, control rats received sesame oil only. After a further 24 h, six animals from each group were given a single intraperitoneal dose of [¹⁴C]NDMA (30 mg/kg body wt., 28 μ Ci/rat) in saline and were killed after 5 h. The livers were removed, cleaned with 0.25 M-sucrose, frozen immediately in liquid N₂ and stored at –80 °C until used. During the course of this study, the food consumed by the animals in both groups was maintained constant.

Nucleic acid and protein isolation

DNA was isolated from individual livers by phenolic extraction procedures (Swann & Magee, 1968). Proteins from samples of the homogenates were precipitated with 10% trichloroacetic acid, and were freed from RNA, DNA and lipid according to the procedure of Schmidt-Tannhauser as described (Munro & Fleck, 1966). The residue, referred to as protein, was dissolved in 0.1 M-NaOH, and a portion of the solution was taken for measurement of radioactivity. Protein content was determined by the method of Lowry *et al.* (1951), and DNA was estimated according to the method of Burton (1956).

Abbreviations used: DHEA, dehydroepiandrosterone; NDMA, dimethylnitrosamine.

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Analytical methods

Isolated DNA was depurinated by adjusting the pH to 1.0 with HCl and incubating at 70 °C for 40 min. A 250 μ l sample of the hydrolysate was applied to the C₁₈ column (0.39 cm $\times$ 30 cm) and 7-methylguanine was resolved by h.p.l.c. using 10 mM-ammonium formate buffer, pH 3.8, as the mobile phase (flow rate 1 ml/min) and monitoring the absorbance of the eluate at 254 nm.

Preparation of microsomes

Microsomes were prepared from control and DHEA-treated rats by differential centrifugation. Briefly, the livers were minced and mixed in 8 vol. of 0.25 M-sucrose containing 10 mM-Tris, pH 7.4. All subsequent operations were conducted at 4 °C. The tissue suspensions were homogenized using a Potter–Elvehjem homogenizer and the homogenates were centrifuged at 10000 *g* for 30 min to remove mitochondria, nuclei and cell debris. The microsomal pellet was obtained by centrifuging the post-mitochondrial supernatant at 105000 *g* for 60 min. The microsomal pellet was suspended in 20 ml of 0.15 M-KCl and re-centrifuged at 105000 *g* for 60 min. The resulting pellet was suspended in 0.25 M-sucrose containing 20% glycerol and stored at –80 °C until used. The cytochrome *P*-450 content was determined according to Omura & Sato (1964).

Microsomal metabolism of NDMA

The incubation medium for NDMA activation contained 100 mM-Hepes buffer (pH 7.4), 2 mM-NADPH, microsomal protein equivalent to 1 nmol of cytochrome *P*-450, 5 mM-semicarbazide and 2 μ M-[¹⁴C]NDMA. The total volume of the mixture was 1 ml. The mixture was incubated at 37 °C for 30 min and the [¹⁴C]formaldehyde generated was determined as previously described (Prasanna *et al.*, 1985).

Metabolism of NDMA *in vivo*

Metabolism of NDMA *in vivo* was determined by the measurement of the rate of exhalation of ¹⁴CO₂ from rats given [¹⁴C]NDMA, as described previously (Magee, 1982).

Statistical analysis

Student's *t* test was used to evaluate the statistical significance of the results.

RESULTS AND DISCUSSION

Following our earlier observation of the inhibition of the binding of 7,12-dimethylbenz[*a*]anthracene to hepatic DNA in rats fed DHEA for 2 weeks (Prasanna *et al.*, 1989), the present investigation was undertaken to study the influence of short-term exposure of rats to DHEA on the initial subcellular effects of the potent hepatocarcinogen NDMA in the liver. As in the earlier studies, short-term exposure of rats to DHEA resulted in significant increases in the liver weight/body weight ratio (0.048 versus 0.058), total liver proteins (including microsomal and cytosolic proteins) and cytochrome *P*-450 (66 versus 143 nmol) when the results are expressed per total liver (Table 1). The DNA content of the livers was not significantly different between the two groups. The enlargement of the liver by DHEA is in agreement with an earlier report (Cleary *et al.*, 1985).

Table 1. Effect of short-term treatment with DHEA on weight, protein content and DNA content of liver in rats

Maintenance and care of the animals are described in the Materials and methods section. Results presented are means $\pm$ s.d. Analyses were conducted in duplicate on samples from individual animals. Each group consisted of six animals. **P* < 0.05, ***P* < 0.001 compared with controls.

	Control	DHEA-treated
Body wt. (g)	229 $\pm$ 9.8	238 $\pm$ 2.6
Liver wt. (wet) (g)	11.1 $\pm$ 1.0	13.8 $\pm$ 0.7**
Total liver protein (mg)		
Homogenate	1715 $\pm$ 256	2163 $\pm$ 273*
Microsomes	104 $\pm$ 11.3	195 $\pm$ 10.8**
Cytosol	662 $\pm$ 72	890 $\pm$ 47**
Total cytochrome <i>P</i> -450 in liver (nmol)	66 $\pm$ 6	143 $\pm$ 8**
Total liver DNA (mg)	39 $\pm$ 0.9	44 $\pm$ 6

Table 2. Effect of DHEA on the binding of [¹⁴C]NDMA to hepatic DNA and proteins *in vivo*

Experimental details are described in the Materials and methods section. Values are means $\pm$ s.d. Six rats were used in each group. Protein is the residue after extraction of lipid and nucleic acids. **P* < 0.05, ***P* < 0.001 compared with controls.

	7-Methylguanine formed (μ mol/mol of guanine)	NDMA bound to total hepatic protein (μ mol)
Control	10480 $\pm$ 1010	5.4 $\pm$ 0.5
DHEA-treated	9200 $\pm$ 390*	7.2 $\pm$ 0.55**

Binding of NDMA to hepatic macromolecules

Data obtained on the binding of NDMA to hepatic DNA and protein are presented in Table 2. In order to assess the damage to DNA by NDMA, the amount of the most frequently measured methylated product of DNA after administration of NDMA, i.e. 7-methylguanine, was determined. The amount of this altered base/mol of guanine was significantly reduced (*P* < 0.05) in DHEA-treated rats. However, the amount of NDMA-derived metabolites binding to total hepatic proteins was higher (1.3-fold) in the steroid-treated rats (*P* < 0.001). These results suggest that DHEA alters the hepatic metabolism of NDMA.

Metabolism of NDMA *in vivo* and *in vitro*

The influence of DHEA on the metabolism of NDMA *in vivo* in rats was further evaluated by the measurement of exhaled ¹⁴CO₂ from rats treated with DHEA. Results presented in Fig. 1 demonstrate that the rate of exhalation of ¹⁴CO₂ is higher in DHEA-treated rats.

Preliminary experiments indicated that the formation of [¹⁴C]formaldehyde from [¹⁴C]NDMA in rat liver microsomes was linear up to 30 min. Hence in all subsequent experiments, a 30 min incubation period was used. A higher rate of [¹⁴C]formaldehyde production by

Fig. 1. Effect of DHEA on the exhalation of CO_2 formed from NDMA

Controls and DHEA-fed rats (four per group) were given intraperitoneal $[^{14}\text{C}]\text{NDMA}$ (30 mg/kg body wt.), and expired $^{14}\text{CO}_2$ was measured after various intervals of time. Values are the means of four determinations. Bars represent range of values obtained. $\blacktriangle$, DHEA; $\bullet$, control.

Table 3. Microsome-mediated metabolism of $[^{14}\text{C}]\text{NDMA}$

Experimental details are given in the Materials and methods section. Values are means $\pm$ S.D. Analyses were conducted in duplicate for each sample. Six animals were used in each group. * $P < 0.01$ compared with control.

Rats	$[^{14}\text{C}]\text{Formaldehyde}$ generated (pmol/30 min per nmol of cytochrome <i>P</i> -450)
Control	104 $\pm$ 16
DHEA-treated	179 $\pm$ 40*

preparations from DHEA-treated rats during the microsomal activation of $[^{14}\text{C}]\text{NDMA}$, when examined at equimolar levels of cytochrome *P*-450 (Table 3) ($P < 0.01$), suggests an increased microsomal activation of NDMA. Additionally, since the total amount of hepatic microsomal cytochrome *P*-450 is higher in DHEA-treated rats (Table 1), the amount of NDMA activated would also be greater. The results presented from *in vivo* and *in vitro* studies, taken together, suggest an enhanced metabolism of NDMA in DHEA-treated rats.

Furthermore, the results of this study indicate that DHEA decreases the methylation of hepatic DNA by NDMA without inhibiting the metabolic activation of the carcinogen. One mechanism by which this might be explained would be that DHEA increases cellular protein content, which in turn serves as a trap for the electrophilic form of NDMA, thus reducing damage to cellular DNA. This is supported in part by an increase in cellular protein content (Table 1). The reported inhibition of glucose 6-phosphate dehydrogenase by DHEA, leading to a presumed decrease in NADPH, has prompted some investigators to suggest that DHEA inhibits the metabolic activation of those carcinogens requiring NADPH for their biotransformation through the mixed function oxidase pathway (Schwartz *et al.*, 1988). However, no significant inhibition of glucose 6-phosphate dehydrogenase by DHEA has been observed *in vivo* (Cleary *et al.*, 1985; Casazza *et al.*, 1986). Additionally, the activities of other enzymes such as isocitrate dehydrogenase and malic enzyme, which are excellent sources for the production of NADPH, are elevated in the livers of DHEA-fed rats (Cleary *et al.*, 1985; Casazza *et al.*, 1986). It should be pointed out that the present data do not distinguish whether DHEA-induced changes in cytochrome *P*-450-mediated activation of NDMA alone are responsible for observed changes in the interaction of NDMA with hepatic macromolecules. Metabolic pathways not involving cytochrome *P*-450 in the bioactivation of NDMA (Phillips *et al.*, 1977) might also play a role in this process. Further studies will be required to separate these potential mechanisms.

Another possibility for the reduction of methylation of DNA by NDMA in DHEA-treated animals could be the alteration in DNA repair. Since the animals in the present study were exposed to NDMA for only a short period of time (5 h), it is unlikely that our results are influenced by this. However, the possibility cannot be ruled out.

The significance of the results of this study is the decreased methylation of DNA in rats treated with DHEA for a short period. The results support the hypothesis that DHEA modulates NDMA-induced hepatocarcinogenesis.

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