BIOCHEMISTRY AND BIOPHYSICS

Relationship between High Sensitivity of Suckling Mice to Hepatocarcinogenic and Antiglucocorticoid Effects of *o*-Aminoazotoluene and Diethylnitrosamine

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Suckling mice were more sensitive to the hepatocarcinogenic effect of various carcinogens compared to adult animals. After treatment with *o*-aminoazotoluene and diethylnitrosamine HNF3-DNA-binding capacity and glucocorticoid-induced liver tyrosine aminotransferase activity in suckling mice decreased more significantly than in adult animals.

Key Words: *hepatocarcinogenesis; transcription factors; enzyme induction; age-specific differences*

Repeated and long-term administration (for several months) of carcinogens is an essential prerequisite to successful induction of liver tumors in adult mice [2], whereas in suckling mice liver tumors develop 8-12 months after single treatment with carcinogens [1]. Disturbances in glucocorticoid regulation of adaptive liver enzymes, including tyrosine aminotransferase (TAT) is an early physiological effect of hepatocarcinogens in adult animals [3]. These changes are most pronounced in animals sensitive to induction of liver tumors, but insignificant in resistant specimens [3]. We found no published data on the influence of hepatocarcinogens on hormonal regulation of TAT activity in 12-15-day-old mice. It should be emphasized that in this period the liver is most sensitive to carcinogens. We compared the effects of *o*-aminoazotoluene (OAT) and diethylnitrosamine (DENA) on glucocorticoid-mediated induction of TAT in suckling and adult mice sensitive to these carcinogens [1].

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MATERIALS AND METHODS

Experiments were performed on male CBA/Lac mice obtained from a vivarium of the Institute of Cytology and Genetics. Parent animals were kept in cages (3 females and 1 male) under natural light/dark conditions and had free access to water and food. OAT (in olive oil) and DENA were injected intraperitoneally to 12-day-old mice in doses of 225 and 100 μg/kg, respectively. The same doses of carcinogens were administered to animals aging 3-4 months. For TAT induction dexamethasone phosphate in a dose of 0.4 mg per 100 g body weight was injected intraperitoneally 19 h after carcinogens. Treated and control mice were decapitated (after 5 h). The livers were taken. Liver TAT activity was measured as described elsewhere [4]. Enzyme activity was expressed in μmol *p*-hydroxyphenylpyruvate formed per 100 mg cytosolic liver protein over 1 h. Protein concentration was measured by the method of Lowry.

In a special series 15 control and 12 OAT-treated mice aging 1.5-2 months were decapitated 18 h after carcinogen administration. Plasma corticosterone con-

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TABLE 1. Effects of OAT and DENA on Basal and Induced TAT Activity in the Liver of Suckling and Adult CBA Mice

Group	Sucklings			Adults		
	μmol *p*-hydroxy-phenylpyruvate/100 mg protein/1 h	% of the control	induc-tion, %	μmol *p*-hydroxy-phenylpyruvate/100 mg protein/1 h	% of the control	induc-tion, %
Basal						
control	25.0±2.1 (5)	100		20.0±0.8 (3)	100	
OAT	40.0±1.6 (4)	160		25.0±1.6 (4)	125	
DENA	43.0±4.5 (5)	172		27.0±2.6 (4)	135	
Dexamethasone-induced						
control	134.0±5.4 (11)	100	536	109.0±4.7 (21)	100	545
OAT	41.0±3.9 (9)*	30.6	103	74.0±4.4 (14)	67.9	296
DENA	49.0±2.6 (9)*	36.6	114	63.0±1.5 (8)	57.8	233

Note. OAT and DENA were administered in single doses of 225 and 100 mg/kg, respectively, 19 h before induction (24 h before decapitation). Number of animals is shown in parentheses. *$p<0.001$ compared to adult animals.

centration was measured by the method of competitive protein binding [10].

The results were analyzed by standard statistical methods. The significance of differences was estimated using Student's *t* test.

HNF3-DNA-binding activity was determined by gel retardation of oligonucleotides corresponding to both chains for binding of this factor in rat transthyretin gene [7]. Electrophoresis was performed after incubation with nuclear extracts of liver cells from control and treated suckling and adult animals. The synthesis and labeling of oligonucleotides, isolation of nuclear extracts, and gel retardation assay were performed as described elsewhere [3].

RESULTS

One day after administration of carcinogens, basal TAT activity in the liver of adult mice increased by 25-35% compared to the control ($p<0.05$, Table 1). In suckling mice receiving carcinogens enzyme activity increased more significantly (by 60-72%). Hence, OAT and DENA in specified doses did not inhibited, but even increased enzyme activity, probably due to irritating effect stimulating the release of catecholamines and glucocorticoids, known genetic inductors of TAT. Eighteen hours after OAT administration blood corticosterone concentration reached 1.50±0.19 μg/100 ml (*vs*. 0.94±0.12 μg/100 ml in the control, $p<0.05$).

Dexamethasone increased TAT activity in control adult and suckling mice more than 5 times. However, in adult and suckling animals receiving carcinogens enzyme activity increased only by 2.3-3 times and 15%, respectively. Hence, pretreatment with OAT suppressed TAT induction in adult and suckling mice by $^1/_3$ (32.1%) and $^2/_3$ (69.4%), respectively. The degree of TAT induction decreased similarly in adult and suckling animals pretreated with DENA (by 41.8 and 63.4%, respectively, Table 1).

Our previous molecular studies showed that hepatocarcinogens decrease DNA-binding activity of transcription factor HNF3γ in liver cells of adult mice. These changes correlate with cell sensitivity to carcinogens that suppress the glucocorticoid-mediated

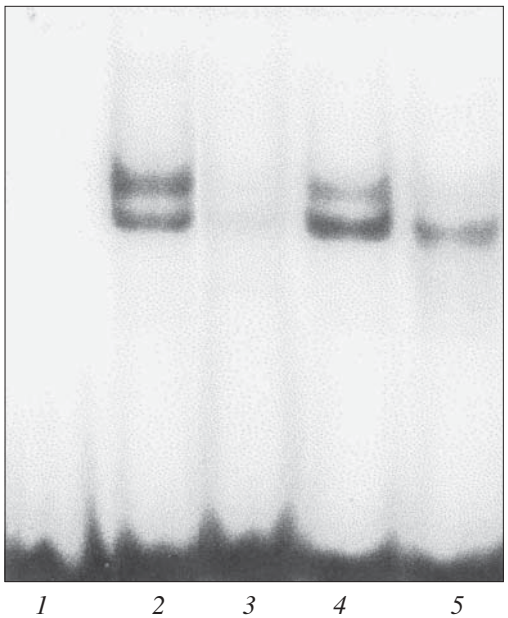

Fig. 1. Effect of *o*-aminoazotoluene (OAT, 225 mg/kg) on DNA-binding activity of HNF3 in nuclear extracts of liver cells from suckling (14-day-old) and adult mice. Mobility of HNF3-binding labeled oligonucleotide probe (*1*); retention of the probe during incubation with various nuclear extracts (*2-4*); control suckling mice (*2*); suckling mice receiving OAT (*3*); control adult mice (*4*); adult mice receiving OAT (*5*). OAT was administered 19 h before decapitation of animals. Extracts were isolated from 3 adult and 15-16 suckling male mice.

induction of TAT and induce tumor growth [3]. Suckling mice exhibited similar characteristics. After treatment with carcinogens, HNF3-DNA-binding activity in liver cell nuclei of suckling mice decreased more significantly than in adult animals (Fig. 1).

Our study confirms our hypothesis [3] that the inhibitory effect of hepatocarcinogens on TAT induction in mice and rats is associated with suppression of HNF3 transcription factors in liver cells. HNF3 proteins determine TAT induction by glucocorticoids [9], play a major role in the appearance and maintenance of differentiated phenotype in definitive liver cells [5,6], and suppress hepatocyte proliferation [8]. It can be hypothesized that the tumor-inducing effect of hepatocarcinogens is related to the inhibition of HNF3 proteins in liver cells.

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