MODIFYING ACTION OF NEONATAL ANDROGENIZATION ON 1,2-DIMETHYLHYDRAZINE-INDUCED CARCINOGENESIS IN MALE CBA MICE

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KEY WORDS: 1,2-dimethylhydrazine; neonatal androgenization; tumors of intestine and pararenal tissue.

The view is generally accepted that androgens secreted during the critical period of development immediately after birth control both differentiation of the hypothalamus—pituitary—gonads system and also differentiation of some of the target organs for steroid hormones [3, 13]. Changes induced by androgens in the neonatal period are long-lasting and irreversible in character. It has been shown that neonatal androgenization in female animals disturbs the hormonal balance [7, 8] and can thereby modify the response to carcinogens in the adult state [2, 5, 8, 15].

Under the influence of 1,2-dimethylhydrazine (DMH) a high frequency of pararenal angiosarcomas is observed in male CBA mice; development of these tumors can be completely prevented by castration and restored by simultaneous injection of testosterone propionate and DMH into castrated males [6].

Accordingly, in the present investigation the possible effect of disturbance or regulation of endogenous hormones due to neonatal androgenization on DMH-induced carcinogenesis was studied in male CBA mice.

EXPERIMENTAL METHOD

On the first day of life a single subcutaneous injection on of 500 μg of testosterone propionate in 0.1 ml of oily solution was given to male CBA mice (177 animals). Three weeks later, 59 (33%) of the androgenized mice were still alive. Starting from the age of 2 months, DMH in distilled water in a dose of 8 mg/kg was injected subcutaneously daily for 20 weeks into 30 androgenized and 30 intact male mice. The corresponding control group consisted of 29 androgenized and 25 intact males. The mice were killed with ether in a state of agony or when palpation revealed tumors, the presence of which was subsequently confirmed at autopsy. All surviving male mice were killed 34 weeks after the beginning of DMH injection. Material was fixed in formalin and subjected to the usual histologic treatment; sections were stained with hematoxylin and eosin. The numerical results were subjected to statistical analysis by the t and chi-square tests.

EXPERIMENTAL RESULTS

The first tumors of the pararenal tissue, which proved to be pararenal angiosarcomas, were found in two neonatally androgenized male mice which died 25 weeks after injection of DMH. Neonatal androgenization increased the frequency of development of these tumors, induced by DMH (Table 1). These tumors in 11 neonatally androgenized animals were large dark red formations, up to 0.5-1 cm in diameter, with a nodular surface, and in 10 animals they were pinpoint structures 2-3 mm in diameter. No metastases were found in any animals. Pararenal angiosarcomas developed bilaterally in 14 mice and unilaterally in eight.

Neonatal androgenization increased the frequency of development of intestinal tumors induced by DMH (Table 1). In five (17.9%) intact and 12 (42.9%, $P < 0.05$) neonatally androgenized males DMH induced carcinoma, whereas in other animals of these groups it induced adenomatous intestinal polyps; the tumors in eight neonatally androgenized mice receiving the

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TABLE 1. Frequency of Tumor Development in Neonatally Androgenized Male CBA Mice Receiving DMH

Experimental conditions	Effective number	Numer of mice with tumors			
		in pararenal tissue	in intestine	in liver	in anal region
Control	24	0	0	0	0
DMH	28	7 (25,0)	32,1 (17,9)	7,1	(10,7)
Androgenization + DMH	28	22 (78,5*)	71,0** (42,9)	25,0	(25,0)
Androgenization	23	0	0	0	0

Legend. Number of mice with malignant tumors (in %) given in parentheses. *P < 0.001, **P < 0.01 compared with DMH.

TABLE 2. Intestinal Tumors in Neonatally Androgenized Male CBA Mice Receiving DMH

Experimental conditions	Effective number	Number of mice with intestinal tumors	Number of mice with solitary intestinal tumors	Number of mice with multiple intestinal tumors	Total number of tumors	Multiplicity index
DMH	28	9 (32,1)	9 (32,1)		9	1
Androgenization + DMH	28	20 (71,4)	12 (42,8)	8 (28,6)	37	1,9

Legend. Number of mice with tumors (in %) given in parentheses.

carcinogen were multiple — from two to five polyps per mouse (Table 2). The frequency of other tumors induced by DMH was unchanged under the influence of neonatal androgenization. DMH caused the development of a hepatoma in three neonatally androgenized and two intact mice, and also the development of a hemangioma of the liver in four neontally androgenized mice. DMH induced the development of carcinoma of the preputial gland in three intact and seven neonatally androgenized males.

A sharp increase in size of the thyroid gland was observed in 17 (74.4%) of 23 male mice subjected to neonatal androgenization only. Microscopic study revealed a papillary-alveolar carcinoma in one case and solid hyperplasia of the thyroid gland in 16 cases. Under the influence of combined treatment with androgen and DMH, no thyroid pathology developed. The writers showed previously that a single injection of androgen into newborn female CBA mice causes a sharp increase in the rate of development and in the frequency of DMH-induced uterine sarcomas [5]. In males, a single injection of testosterone propionate caused an increase in the frequency of pararenal angiosarcomas and intestinal tumors induced by DMH. Several communications [4, 6, 14] mentioned the importance of hormonal factors for the development of DMH-induced tumors in these situations. It can accordingly be postulated that neonatal injection of androgen caused changes in hormonal regulation in the males leading to the more frequent development of these tumors. Data in the literature on the fall in the testosterone level in males after a single injection of a high dose of androgen in the neonatal period are scanty [1]. However, we know that one manifestation of the effect of androgen on sexual differentiation of the hypothalamus is the presence of sex differences in steroid hormone metabolism, interpreted as neonatal imprinting [3]. A single injection of testosterone propionate into newborn males may perhaps intensify sexual differentiation of testosterone metabolism in these animals and may induce changes in characteristics of their testosterone dynamics such as the level of metabolic clearance and the biological half-life of the androgen in the circulating blood [9]. Since injection of androgen in the critical period of development induces long-lasting changes in active enzyme systems not only in the hypothalamus and liver of neonatal animals, but also in other organs [10], a single dose of testosterone may thus lead to changes in steroid metabolism, as a result of which there is a change in the frequency of pararenal angiosarcomas and intestinal tumors induced by DMH in male CBA mice. On the basis of data obtained by other workers [11, 12] and showing that in-

jection of androgen during the first days of life modifies the sensitivity of enzyme systems and tissues to external factors, it can also be postulated that a single injection of testosterone propionate in the critical period of sexual differentiation of the hypothalamus enhanced the reactivity of the pararenal and intestinal tissue to DMH.

Neonatal androgenization caused thyroid pathology in 74% of male CBA mice. No precise explanation of this phenomenon can be given, but it can be tentatively suggested that a single injection of testosterone propionate into day-old animals can cause a breakdown of hypothalamic regulation of thyroid function in male CBA mice.

Neonatal androgenization thus has a modifying action on the development of DMH-induced pararenal angiosarcomas and intestinal tumors in male CBA mice.

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HUMAN TUMOR STRAINS TRANSPLANTABLE INTO NUDE MICE AND RATS

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This paper described the use of strains of carcinoma of the lung (CL-3) and of the larynx (CLar), Ewing's sarcoma (ES), fibrosarcoma (FS), Wilms' tumor (WI), and carcinoma of the kidney (CK) for transplantation into nude mice and rats. The above-mentioned strains are part of a collection of transplantable human tumors at the All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR.

EXPERIMENTAL METHOD

Nude mice aged 6-8 weeks and nude rats aged 4-6 weeks, reared at the Institute, were used for transplantation. Strains CL-3, CLar, WT, and CK were obtained from material taken at operations. The tumor was transplanted in fragments into the mice. Strains ES and FS were obtained by transplantation of human tumor cell lines from tissue culture [4]. In that case 10^6 cells in 0.5 ml of medium were injected subcutaneously into mice. A suspension of human tumors after serial passages throughmice was injected into the rats. The suspension contained 150 mg tissue in 0.5 ml. Subsequent serial transplantations in both rats and mice were carried out with tumor suspension.

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