Effect of Neonatal Injection of Sodium Glutamate and Diethylnitrosamine on Hepatocarcinogenesis, Reproductive and Adrenocortical Systems of Male Mice

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Neonatal injection of sodium glutamate before injection of diethylnitrosamine decreased the number of tumor nodes in the liver of male mice, decreased the weight of the testes and adrenals and blood level of testosterone (but increased blood level of corticosterone), impaired recovery of diethylnitrosamine-disturbed sexual motivation in half of males. Anticarcinogenic effect of sodium glutamate is explained by feminization of males under its effect.

Key Words: *sodium glutamate; diethylnitrosamine; hepatocarcinogenesis; sexual motivation*

Neonatal injection of hepatocarcinogens, including diethylnitrosamine, more often induces liver tumors in males than in females [1]. This is believed to be due to involvement of male sex hormones in carcinogenesis processes [6,7].

Neonatal injection of sodium glutamate suppresses reproductive function in mice and rats. Decreased fertility and reduced weight of testes, low levels of follicle-stimulating hormone and testosterone in the plasma are characteristic of these males. We hypothesized that injection of sodium glutamate during the early ontogeny would modify chemical carcinogenesis induced by diethylnitrosamine in mouse liver by modulating the testosterone status of males. It was also important to evaluate the effects of carcinogen and glutamate+diethylnitrosamine on the male reproductive function. Activation of the pituitary-adrenal axis is a primary response to any stress factor [5], therefore we investigated the effect of sodium glutamate and diethylnitrosamine on the adrenocortical system.

We studied the effect of neonatal injection of sodium glutamate on chemical hepatocarcinogenesis induced by injection of diethylnitrosamine and on the reproductive and adrenocortical systems in male mice with experimentally induced carcinogenesis.

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MATERIALS AND METHODS

Experiments were carried out on CBA/LacSto mice from the vivarium of Institute of Cytology and Genetics. The animals were kept in groups (3 females and 1 male per cage) under conditions of natural illumination on PK 120-1 granulated fodder (Informkorm) with free access to water. Newborn mice were divided into 3 groups. Group 1 mice remained intact. Group 2 animals were intraperitoneally injected with 0.05 mg/g diethylnitrosamine (DENA; Serva) at the age of 12 days. Group 3 animals intraperitoneally received 2 mg/g sodium glutamate (Aldrich, 8% solution) on days 1, 3, 5, 7, and 9 of life, and DENA (0.05 mg/g) at the age of 12 days. After 2 weeks the animals were separated from their parents and were kept in small groups until the end of the experiment.

At the age of 10 months the males were tested for sexual motivation; it was evaluated by the time spent near the wall behind which a receptive female was placed [2]. Three days before testing the males were placed alone into 28×14×10 cm cages divided into two

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equal parts with a plastic wall with holes. On the day of testing an estrous female was placed in the empty compartment behind the wall. The male could see and smell the female, but could not directly contact with it. Testing was carried out for 10 min after the female was placed into the cage. The males remaining alone in cages with an empty neighboring section served as controls. The behavior of males was videotaped. Estrus in females was induced by intraperitoneal injection of 10 U chorionic gonadotropin (Serono) 24 h before testing.

After 20-min testing the males were rapidly decapitated. Plasma testosterone was radioimmunoassayed and corticosterone was measured by competitive protein binding.

The liver was fixed in 10% formalin and the number and size of surface tumor nodules of 1 mm and more in diameter were evaluated under a binocular magnifying glass with an ocular micrometer.

The results were statistically processed using Statistica 6 software. The significance of differences between the groups was evaluated using Mann—Whitney test, the correlation between the signs was evaluated using the Spearman method.

RESULTS

No tumors in the liver were found of intact males. Multiple tumor formations in the liver (50.7±4.8 tumor node/mouse) were found in all male mice injected with DENA. In the females DENA induced only 3.6± 0.8 tumor nodes/liver. The results are in line with published data [1].

Examination of the liver showed that in animals injected with sodium glutamate before injection of DENA the number of tumor nodes was almost 3-fold lower than in males injected with DENA alone. The diameter of the largest tumor and the number of large tumors (2 mm in diameter) were also lower in animals injected with sodium glutamate. These data suggest that neonatal injection of sodium glutamate had a pronounced anticarcinogenic effect on males, the sensitivity to the carcinogen in these animals was close to that in females.

Changes in the reproductive system of males treated with sodium glutamate attest to their feminization. The weight of testes and blood level of testosterone were lower in this group compared to intact mice and animals injected with DENA alone (Fig. 1). One testicle was completely reduced in 7 of 32 males in this group. No animals with one testicle were detected among intact animals and animals injected with DENA alone.

The weight of the adrenals and blood corticosterone level were evaluated in all animals. The weight of adrenals was decreased in mice injected with DENA and even more so in animals injected with sodium glutamate before DENA (1.5 times less than in intact animals), but blood corticosterone concentration in them 1.5-fold surpassed that in intact mice (Fig. 2).

The rats treated with sodium glutamate exhibited signs of adrenal hyperfunction [3]. The central injury caused by sodium glutamate destroyed the regulation of the hypothalamic-pituitary-adrenal axis. Our findings attest to activation of the adrenocortical system in mice treated with sodium glutamate before induction of carcinogenesis. Hyperactivity of the adrenal system after sodium glutamate treatment was observed in young (according to previous report [3]) and old (in our experiment) mice, that is, high activity of the adrenal system persisted throughout the life.

Decreased level of testosterone and low weight of testes detected in our study and complete reduction of

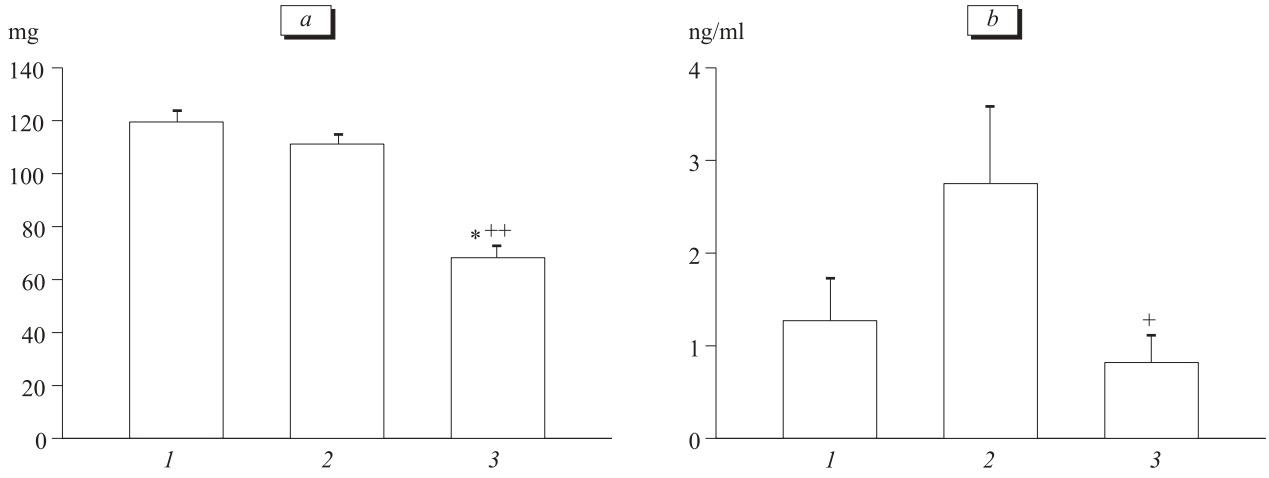

Fig. 1. Effect of neonatal injection of diethylnitrosamine (DENA) and sodium glutamate+DENA on testicular weight (*a*) and blood testosterone concentration (*b*) in male mice. Here and in Fig. 2: *1*) intact mice; *2*) DENA injection; *3*) glutamate+DENA. $^{*}p<0.001$ compared to intact animals; $^{+}p<0.05$, $^{++}p<0.001$ compared to mice injected with DENA.

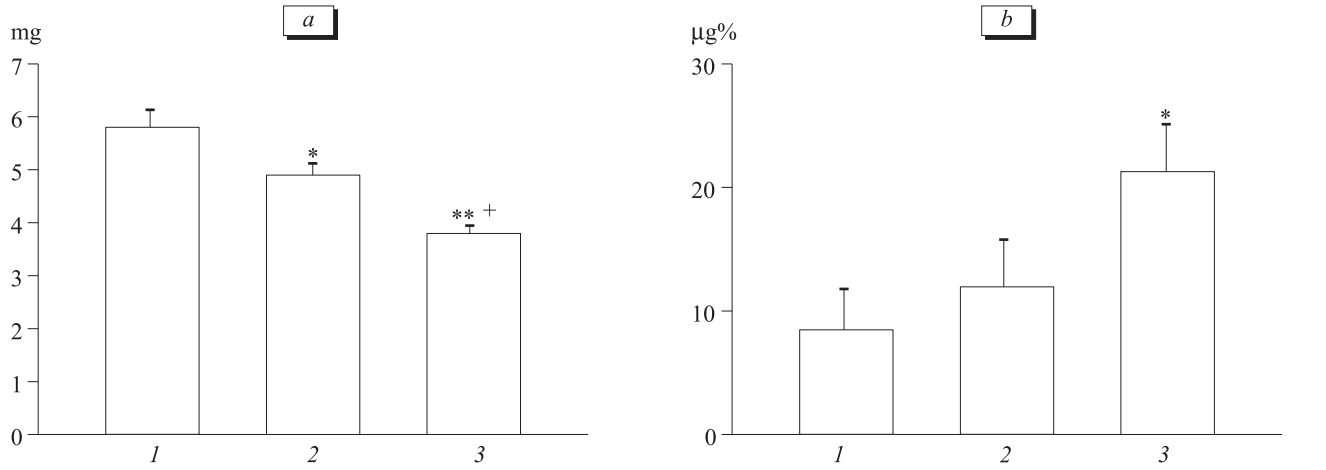

Fig. 2. Effect of neonatal injection of DENA and sodium glutamate followed by DENA on adrenal weight (*a*) and concentration of corticosterone (*b*) in the blood of males. **p*<0.05, ***p*<0.001 compared to intact animals; +*p*<0.001 compared to mice injected with DENA.

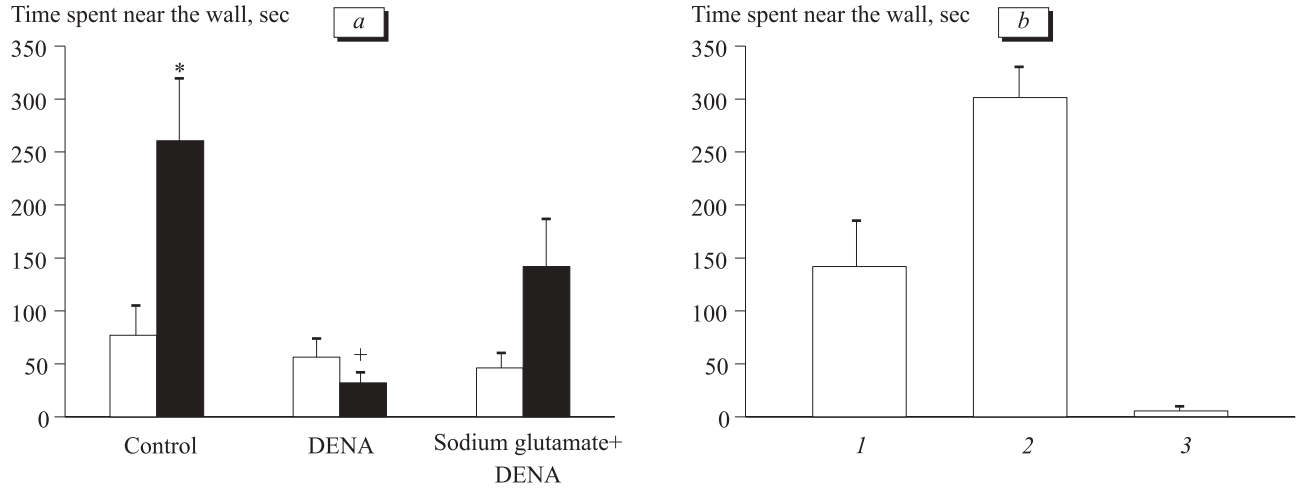

Fig. 3. Effect of DENA on sexual motivation of males. *a*) effects of DENA and DENA+sodium glutamate on sexual motivation. Light bars: empty section of the cage; dark bars: section with a female. *b*) all males treated by sodium glutamate+DENA (*1*); males exhibiting (*2*) and not exhibiting (*3*) sexual motivation. **p*<0.01 compared to the empty section of the cage, +*p*<0.05 compared to the control.

one testicle in some males treated with sodium glutamate can be attributed to high level of corticosterone in these animals. Some data indicate that high level of corticosterone in rats induces apoptosis of Leydig cell [4].

Presentation of a receptive female to intact male 3-fold prolonged the time spent near the wall behind which the female was placed in comparison with the empty section of the cage. DENA suppressed sexual motivation in males, the activating effect of the female was not observed. The animals injected with sodium glutamate before DENA spent less time near the wall with the female behind it in comparison with animals with preserved sexual motivation, but more time in comparison with intact animals (empty compartment). The animals of this group were clearly divided into two subgroups by sexual motivation. In 6 of 13 males the level of sexual motivation was comparable to that of controls, while in 7 others sexual motivation was completely absent (Fig. 3), which attests to different degree of feminization of males treated with sodium glutamate.

Blood testosterone level increased in intact males after 20 min spent in one cage with the female ($p<0.01$). Testosterone level did not change in response to presentation of a female in animals injected with DENA and sodium glutamate before DENA. In none animal presentation of the female changed the level of corticosterone.

No differences in testosterone and corticosterone levels in subgroups with normal sexual motivation and without sexual motivation were detected in the males treated with sodium glutamate before DENA injection. However, these subgroups differed significantly by the number of tumors in the liver. A significant positive correlation was detected between the degree of sexual motivation in males treated with sodium glutamate

and tumor development in the liver of these males, between the time spent near the wall and total number of tumors (r=0.8, p<0.001), time and number of tumors >2 mm in diameter (r=0.8, p<0.001), time and maximum diameter of tumor (r=0.8, p<0.001).

The animals treated with sodium glutamate before DENA were divided into 2 subgroups by many parameters. In one subgroup males had much more tumors in the liver and the relative weight of the adrenals and testicles was less than in the other subgroup, sexual motivation was preserved in subgroup 1 and absent in subgroup 2. This suggests that greater or lesser feminization of males under the effect of glutamate was to a certain measure responsible for tumor development in the liver. Anticarcinogenic effect of sodium glutamate was more pronounced in animals without sexual motivation, and more pronounced feminization. As neither basal testosterone level, nor its level during sexual activation in the presence of female did not differ in subgroups with and without sexual motivation, we hypothesize that sodium glutamate modified carcinogenesis not only by modulating the testosterone status of males. Presumably, its effect was realized also through central mechanisms and other hormonal systems apart from the hypothalamic-pituitary-testicular system.

Hence, neonatal injection of sodium glutamate protected mice from chemically induced carcinogenesis. The number of developing tumors decreased 3-fold, the number of large tumors decreased, and the maximum diameter of tumors also decreased. Adrenocortical function was modified: adrenal weight decreased, while blood corticosterone level increased. Injection of sodium glutamate restored the sexual motivation to the normal level in half of males injected with DENA, but did not normalize the pattern of sexual stimulation impaired by DENA, because the level of testosterone did not increase in response to presentation of a female. The positive correlation between the degree of sexual motivation and total number of tumors in the liver, number of large tumors, and maximum diameter of tumor indicates that the protective effect of sodium glutamate in hepatocarcinogenesis is based on feminization of males.

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