EFFECT OF POSTNATAL PERSISTENT ESTRUS SYNDROME ON REALIZATION OF THE MULTIGENERATION CARCINOGENIC EFFECT OF N-NITROSOMETHYLUREA

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There is evidence that various modifying factors may have opposite effects in the postnatal period on the realization of transplacental carcinogenesis induced by nitroso compounds [1-4]. Attempts have been made to modify multigeneration carcinogenesis induced transplacentally in animals of not only the first (F_1), but also the second (F_2) generations [5, 7]. Induction of the persistent estrus syndrome in young sexually mature F_1 rats exposed to the transplacental action of 7,12-dimethylbenz(a)anthracene (DMBA) or N-nitrosomethylurea (NMU) led to intensification of carcinogenesis [1, 8]. However, the use of this same modifying factor on postnatal F_2 females has no significant influence on realization of the multigeneration effect of DMBA [8].

The aim of this investigation was to study the effect of a persistent estrus syndrome induced postnatally in female rats of two generations on realization of the multigeneration carcinogenic effect of another carcinogen, namely NMU.

EXPERIMENTAL METHOD

Albino rats aged 3 months and weighing 200 g were obtained from the "Rappolovo" Nursery, Academy of Medical Sciences of the USSR. On the second day of pregnancy the rats were given a single intraperitoneal injection of NMU in a dose of 20 mg/kg. The F_1 females were divided into two groups. Animals of group 1 were subjected to no further procedures during the postnatal period. Animals of group 2 were ovariectomized at the age of 3 months and one ovary was autografted beneath the skin of the tail, leading subsequently to the development of a persistent estrus syndrome in these animals [9]. Some of the F_1 females were crossed with males whose mothers also had been exposed to NMU during pregnancy, in order to obtain F_2 offspring. F_2 females of group 3 were subjected to no further procedures, whereas F_2 females of group 4 underwent the same operation as the F_1 animals of group 2. Female rats of group 5 were not exposed to the transplacental action of NMU, but a persistent estrus syndrome was induced in them in the postnatal period. Animals of group 6 were subjected to no procedure of any kind and served as the intact control. The animals remained under observation until natural death. All rats which died or were killed in a feeble state by inhalation of ether vapor were autopsied and their internal organs, including brain and spinal cord, and also the cranial and principal peripheral nerve trunks, if there was any suspicion of tumor growth, were fixed in Kaiserling's fluid. Material for histological investigation was embedded in paraffin wax and sections cut to a thickness of 5-7 μ were subjected to statistical analysis by Student's t test, the chi-square test, and the method in [10].

EXPERIMENTAL RESULTS

Neoplasms developed in F_1 females (group 1) exposed to the transplacental action of NMU in 60% of cases (Table 1). Malignant tumors of the peripheral nervous system (PNS; 30%) and also of the kidneys (15.0%) were found most frequently, but were not detected in females of the control group 6. The average latent period of development of all tumors in the animals of group 1 was significantly shorter than in the control (495 and 693 days respectively; $p < 0.01$).

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TABLE 1. Frequency and Location of Neoplasms in Female Rats of Two Consecutive Generations after Transplacental Exposure to NMU and Induction of Postnatal Persistent Estrus Syndrome (PES)

Group of animals	Character of procedure and generation of animals	Effective number of rats	Number of rats with tumors	Total number of tumors	Number of rats					Mean life span of rats, days
					with malignant tumors of the				with tumors in other locations	
					CNS	PNS	kidneys	uterus		
1	NMU; F_1	40	24 (60,0%)	27	1 (2,5%)	12 (30,0%)	6 (15,0%)	1 (2,5%)	6 (15,0%)	500
2	NMU + PES; F_1	36	26 (72,2%)	36	9 (25,0%)	11 (30,0%)	5 (13,9%)	—	10 (27,8%)	482
3	F_2	31	11 (35,5%)	15	2 (6,5%)	1 (3,2%)	1 (3,2%)	—	10 (31,3%)	608
4	PES; F_2	31	8 (25,8%)	8	1 (3,2%)	—	—	—	7 (21,6%)	464
5	PES	42	17 (40,5%)	18	—	—	—	2 (4,8%)	15 (35,7%)	527
6 (control)	—	50	18 (36,0%)	23	—	—	—	—	18 (36,0%)	734

Legend. Malignant tumors: PNS — neurinomas; uterus — sarcoma (group 1), leiomyoma (group 5); CNS: in group 1 — mixed oligoastrocytoma, in group 2 — oligodendroglioma (8) and meningioma (1), in group 3 — oligodendroglioma (1) and mixed oligoastrocytoma (1) in group 4 — oligodendroglioma; of the kidneys: in group 1 — mesenchymal tumor (6), in group 2 — mesenchymal tumor (3), solid carcinoma (1), and papillary adenocarcinoma (1), in group 3 — mesenchymal tumor (1). Malignant tumors in other locations: group 1 — pituitary adenoma (1), thyroid adenoma (1), follicular thyroid carcinoma (1), thecoma of the ovary (1), fibroadenoma of the mammary gland (3); group 2 — pituitary adenoma (2), fibrous histiocytoma of the skin (4), intestinal adenoma (2), fibroadenoma of the mammary gland (3); group 3 — pituitary adenoma (3), thecoma of the ovary (1), medullary vaginal carcinoma (1) corticosteroma (1), fibroadenoma of the mammary gland (5); group 4 — pituitary adenoma (4), adrenal adenoma (1), fibroadenoma of the mammary gland (2); group 5 — pituitary adenoma (7), intestinal adenoma (1), thecoma of the ovary (1), leiomyosarcoma of the vagina (1), anaplastic lung cancer (2), fibroadenoma of the mammary gland (4); group 6 — pituitary adenoma (10), pituitary adenocarcinoma (1), thyroid adenoma (1), anaplastic lung cancer (1), phaeochromocytoma (1), fibroadenoma of the mammary gland (8), adenocarcinoma of the mammary gland (1). *$p < 0.05$ compared with group 1.

In the rats of group 2, exposed to the transplacental action of NMU, and in which a persistent estrus syndrome was induced in the postnatal period, the total frequency of tumor development did not differ significantly from that in the females of group 1. However, tumors developed in their CNS more often than in rats exposed to the transplacental action of NMU only (25.0 and 2.5% respectively; $p < 0.01$). Tumors of the kidneys developed in the animals of these two groups with equal frequency. Incidentally, in the rats of group 2 (in 4 cases) malignant tumors of the skin were found, although these did not develop in the females of group 1, together with neoplasms characteristic of animals with the persistent estrus syndrome (group 5). The mean time of discovery of tumors in this group (385 days) was significantly shorter than in the rats of groups 1 and 5 (495 and 484 days respectively; $p < 0.01$).

Postnatal induction of persistent estrus in rats, which is accompanied by various hormonal disturbances [9], thus leads to intensification of transplacental carcinogenesis induced by NMU; this is expressed primarily as an increase in the frequency of development of tumors in the CNS. Similar results were obtained previously [1]. When another carcinogen (DMBA) was used, induction of persistent estrus in rats also increased the frequency of development of tumors, mainly of the nervous system and mammary gland [8].

The total frequency of tumors in the F_2 rats (group 3) did not differ significantly from the control. Nevertheless, the development of tumors in the nervous system (oligodendroglioma, mixed oligoastrocytoma, and malignant neurinoma) and of a mesenchymal tumor of the kidney in these animals in 9.7% of cases is evidence of manifestation of the multi-

generation effect of NMU. In F_2 females, with postnatal induction of the persistent estrus syndrome (group 4), tumors developed with the same frequency (25.8%) as in the rats of group 3. With the exception of tumors in the nervous system (oligodendroglioma) the remaining neoplasms which developed in the animals of group 4 were also characteristic of the spontaneous background in animals of this breed.

Induction of the persistent estrus syndrome in F_2 animals thus had no significant effect on realization of the carcinogenic effect of NMU in the second generation. This may be connected with the fact that some F_2 animals with hereditary predisposition to tumors, after developing hormonal disturbances as a result of induction of a persistant estrus syndrome in them, were found to be less viable and died at an earlier age, i.e., even before they developed tumors. Nevertheless, induction of the persistent estrus syndrome in the F_1 females in the postnatal period is a promoting factor in the development of tumors of the nervous system induced transplacentally by both DMBA and NMU.

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