DISTRIBUTION OF CARCINOGENIC DIALKYLHYDRAZINES-^{3}H IN THE NEUROENDOCRINE SYSTEM AND THEIR ANTIGONADOTROPIC EFFECT IN RATS

V. N. Anisimov, M. A. Azarova, A. Yu. Dmitrievskaya, A. Ya. Likhachev, A. S. Petrov, and Ya. D. Shaposhnikov

UDC 615.277.4.033.814

Three hours after injection of dimethylhydrazine (DMH)-^{3}H into male rats the specific radioactivities of the pituitary, adrenal cortex, and testes were 53, 85, and 108% higher, respectively, than of the liver. Incorporation of the isotope into the hypothalamus was 33% greater than into the liver, and 69 and 44% greater, respectively, than into the cerebral cortex and brain stem. Three hours after injection of diethylhydrazine (DEH)-^{3}H into male rats the specific radioactivities of the pituitary and testes were 57 and 108% higher, respectively, than that of the liver. A single injection of DMH or DEH in a dose of 21 mg/kg into male rats led to a significant decrease in the content of follicle-stimulating hormone (FSH) in the pituitary (by 63 and 53%, respectively). The results are evidence that carcinogenic dialkylhydrazines have a marked effect on the neuroendocrine system in rats.

KEY WORDS: 1,2-dialkylhydrazines; neuroendocrine system; antigonadotropic action.

Several hydrazine derivatives and, in particular, 1,2-dimethylhydrazine (DMH) and 1,2-diethylhydrazine (DEH) are known to have powerful carcinogenic activity. For instance, DMH induces tumors of the intestine selectively in rats and with high frequency [7, 11], whereas DEH induces tumors of the nervous system, liver, and mammary gland and leukemias [10]. The basic role in the mechanism of the carcinogenic effect of these dialkylhydrazines is played by methylation of nucleic acids and proteins of the target tissues [9]. Meanwhile, evidence has been obtained of the selective action of DMH on the biogenic amine level in the hypothalamus of rats and of the development of various hormonal hypermetabolic disturbances in these animals favoring tumor growth [3, 4].

There is no information in the literature on the effect of DEH on the neuroendocrine system. In this connection it seemed interesting to compare the action of these carcinogens with their affinity for different organs on the gonadotropic activity of the pituitary and to study their distribution in the neuroendocrine systems of rats.

EXPERIMENTAL METHOD

Male rats bred at the "Rappolovo" nursery, Academy of Medical Sciences of the USSR, weighing 150-170 g were used. DMH-^{3}H or DEH-^{3}H (specific activity 12 mCi/g)* was injected subcutaneously in a dose of 21 mg/kg, as described previously [9]. Three hours after injection of the carcinogen, when the blood radioactivity was at its highest [9], the animals were decapitated. Pieces of tissue from various organs (pituitary,

*The authors are grateful to Yu. M. Kapustin for providing the tritium-labeled carcinogens.

Laboratory of Endocrinology, Laboratory of Experimental Tumors, Laboratory of Biochemistry, and Laboratory of Organic Synthesis, N. N. Petrov Research Institute of Oncology, Ministry of Health of the USSR, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR L. M. Shabad.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 82, No. 12, pp. 1473-1475, December, 1976. Original article submitted June 4, 1976.

TABLE 1. Distribution of Tritium in Various Organs of Male Rats 3 h after Injection of DMH-^{3}H or DEH-^{3}H (M ± m)*

Organ	Specific radioactivity (counts/min per g wet tissue)	
	DMH-^{3}H	DEH-^{3}H
Liver	520±106	388±53†
Pituitary	797±85†	611±72†
Adrenals	961±113‡	495±44
Testes	1083±109†	806±149†
Brain:		
cerebral cortex	411±51‡	313±13
stem	483±65‡	227±15†
hypothalamus	694±72	286±35

*Mean values from 5 experiments given.
†Difference from specific radioactivity of liver significant ($P < 0.05$).
‡Difference from specific radioactivity of hypothalamus significant ($P < 0.05$).

adrenals, testes, cerebral cortex, brain stem, hypothalamus, and liver) were weighed and mixed with 0.3 ml NCS tissue solvent (Radiochemical Centre, Amersham, England). The tissues dissolved completely on incubation for 20 h at 5°C. After cooling to room temperature the solution was partly neutralized by the addition of 0.03 ml glacial acetic acid. The resulting solution was mixed with 3 ml ethylcellosolve, and 5 ml of a scintillation solution containing 6 g PPO and 75 mg POPOP (both from Radiochemical Centre, Amersham, England) to 1 liter toluene was added. Radioactivity was counted in a liquid scintillation counter. The counting efficiency was determined for each sample by means of the counter computer followed by scaling against a standard curve. In a special experiment nonradioactive DMH and DEH were injected in a dose of 21 mg/kg into male rats and their pituitary follicle-stimulating activity was determined 7 days later by a biological method [13], using NIH-FSH-S_3 as the standard. The results were subjected to statistical analysis with the aid of Student's t and Wilcoxon's U criteria.

EXPERIMENTAL RESULTS

As the results given in Table 1 show, 3 h after injection of DMH-^{3}H the specific radioactivity of the tissue of the endocrine glands and hypothalamus was higher than that of the liver, cerebral cortex, and brain stem. After injection of DEH-^{3}H, maximal radioactivity was found in the tissue of the testes and pituitary gland.

Both DMH and DEH significantly lowered the FSH content in the pituitary of the rats 1 week after a single injection of the compound (by 63 and 53%, respectively; $P < 0.05$) (Fig. 1).

Earlier investigations [3, 8] showed that 1 or 2 injections of DMH into rats lower the gonadotropic activity of the pituitary in both males and females. Prolonged injection of this carcinogen led to atrophy of the testes in males and blocked compensatory hypertrophy of the ovary in hemicastrated females. No information on the effect of DEH on pituitary gonadotropic function in rats could be found in the literature. Druckrey et al. [10], however, observed the development of adenocarcinomas of the mammary gland in 75% of females receiving DEH. The fact that high specific radioactivity of the tissues after injection of both dialkylhydrazines-^{3}H was discovered in the testes and pituitary (for DMH by 108 and 53% respectively higher than in the liver, and for DEH by 108 and 57% higher respectively than in the liver) suggests that these carinogens have a direct action on these endocrine glands. At the same time, the fact will be noted that in experiments with DMH-^{3}H incorporation of the label into the hypothalamic tissue was at a considerably higher level than into the tissue of the cerebral cortex or brain stem (by 69 and 44%, respectively). These results are in good agreement with the selective modification of the level of biogenic amines in the rat hypothalamus demonstrated previously by DMH [4]. The pituitary gonadotropic function is under the regulatory influence of the corresponding hypothalamic centers [6]. The antigonadotropic effect of DMH is evidently exerted directly at the hypothalamic level also. At the same time, the results do not rule out completely a central component in the mechanisms of the antigonadotropic action of DEH, for incorporation of the label into the tissues of the brain, the target organ for DEH, was only negligibly less than into the tissues of the liver, the organ which metabolizes the carcinogen and is also its target organ. Since DMH and DEH have selective carcinogenic activity against

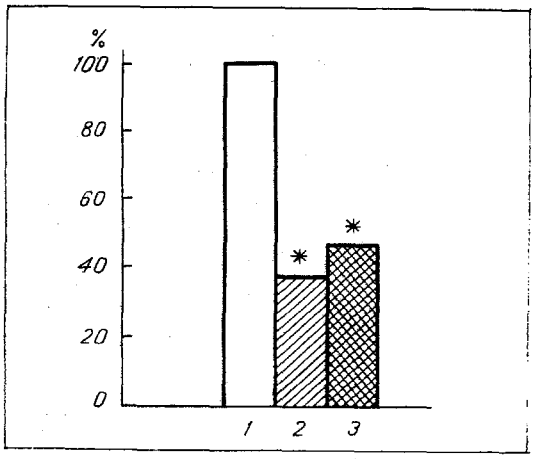

Fig. 1. FSH content in pituitary of male rats 1 week after a single injection of DMH or DEH in a dose of 21 mg/kg (in % of control): 1) control; 2) after DMH; 3) after DEH. *) Difference from control significant ($P < 0.05$). Pituitary glands from 10 rats were tested in each group.

different tissues, it can be postulated that the similarity between their antigonadotropic action, revealed in the present experiments, is due to the ability of hydrazine derivatives to disturb catecholamine biosynthesis [4, 12]. At the same time, it has been shown that disturbance of adrenergic transmission in hypothalamic structures controlling pituitary gonadotropic function plays an important role in the mechanism of elevation of the threshold of sensitivity of the hypothalamus to homeostatic inhibition [1]. There is evidence that elevation of the hypothalamic threshold of sensitivity to inhibition lies at the basis of the materialization of the neuroendocrine program of development and aging and that it creates favorable conditions for the formation of age pathology and, in particular, it promotes the development of neoplasms [5]. It was shown previously that the phenomenon of elevation of the hypothalamic threshold in rats develops soon after injection of both DMH and other carcinogens of different classes [2, 3, 8].

It can therefore be postulated that, besides their specific action on the target tissues, in which the tumors actually arise, another important factor in the production of the carcinogenic effect of dialkylhydrazines is their influence on the neuroendocrine system, with the creation of conditions promoting proliferation of cells injured by the carcinogen.

LITERATURE CITED

1. V. N. Anisimov, Byull. Éksp. Biol. Med., No. 12, 44 (1975).
2. V. N. Anisimov and V. M. Dil'man, Vopr. Onkol., No. 5, 61 (1974).
3. V. N. Anisimov, E. G. L'vovich, I. A. Vasil'eva, et al., in: Carcinogenic N-Nitroso Compounds – Action. Synthesis, Determination. Proceedings of the 2nd Symposium [in Russian], Tallin (1975), pp. 12-14.
4. V. N. Anisimov, V. K. Pozdeev, A. Yu. Dmitrievskaya, et al., Byull. Éksp. Biol. Med., No. 11, 1359 (1976).
5. V. M. Dil'man, Endocrinological Oncology [in Russian], Leningrad (1974).
6. J. Szentagothai et al., Hypothalamic Control of the Anterior Pituitary [in Russian], Budapest (1965).
7. K. M. Pozharisski (K. M. Pozharisskii), in: Pathology of Tumors in Laboratory Animals (ed. by V. S. Turusov), Vol. 1, Part 1, Lyon (1973), pp. 119-140.
8. K. M. Pozharisskii and V. N. Anisimov, Patol. Fiziol., No. 1, 47 (1975).
9. K. M. Pozharisski (K. M. Pozharisskii) et al., Int. J. Cancer, 15, 673 (1975).
10. H. Druckrey et al., Naturwissenschaften, 53, 557 (1966).
11. H. Druckrey et al., Naturwissenschaften, 54, 285 (1967).
12. "Editorial," Lancet, 1, 979 (1973).
13. S. Steelman and F. M. Pohley, Endocrinology, 53, 604 (1953).