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EFFECT OF SEX HORMONES ON 1,2-DIMETHYLHYDRAZINE METABOLISM IN THE CBA MOUSE KIDNEY

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The mechanisms responsible for the manifestation of sexual dimorphism in carcinogenesis have not been adequately studied. Convenient models for their investigation are sarcoma of the renal capsule and adenomas of the kidneys, which can be induced with high frequency in male CBA mice by 1,2-dimethylhydrazine (DMH) [2] but which appear only infrequently in females under the same conditions [1]. Preliminary castration of males sharply reduces the frequency of formation of these tumors [3], while testosterone propionate (TP) restores their frequency to its initial level, but only if it is administered simultaneously with DMH [4]. DMH is a carcinogen with indirect action, requiring metabolic activation in the recipient organism; one cause of differences in the sensitivity of male and female CBA mice to the action of this carcinogen may therefore be a difference in the activity of its metabolism in the target organ.

This paper describes the study of DMH metabolism in the kidneys and liver of CBA mice.

EXPERIMENTAL METHOD

Male and female CBA mice aged 2-3 months were obtained from the "Stolbovaya" nursery, Academy of Medical Sciences of the USSR. The males were castrated 2 weeks before the experiment. TP was injected subcutaneously in olive oil in a sessional dose of 0.5 mg per mouse 24 h before the experiment. To determine incorporation of ^{14}C-DMH into DNA in the mouse liver and kidneys, unlabeled DMH·2HCl was added to the ^{14}C-DMH.2HCl (specific radioactivity 8.4 mCi/mmole, from New England Nuclear, USA) until the specific radioactivity was 3.5 mCi/mmole (the pH was adjusted to physiological with dry Na_2CO_3), and the product was injected subcutaneously in a dose of 15 mg/kg body weight (calculated as base). The ^{14}C-DMH was generously provided by the International Cancer Research Agency (Lyon, France).

The microsomal fraction was isolated by the method in [7]. To determine the demethylating activity of the microsomes, the substrate (10 mM) was incubated for 5 min at 37°C in a mixture containing 3 mM NADPH (NADH), 1 mg/ml microsomal protein, 16 mM $MgCl_2$, and 0.1M phosphate buffer, pH 7.4. The reaction was stopped by the addition of 0.5 ml of 20% TCA. After precipitation of the protein by centrifugation for 10 min at 3500 g the formaldehyde concentration in the supernatant was determined by the color reaction described in [9]. To isolate DNA the separate organs were frozen in liquid nitrogen and kept at −20°C for not

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TABLE 1. Velocity of N-Demethylation of DMH and AP (in μmoles/30 min/mg protein) in Microsomal Enzyme System of the Kidneys and Liver of CBA Mice (M ± m)

Group of animals	Kidneys		Liver	
	DMH	AP	DMH	AP
Males	25,4±1,4	22,3±1,6	10,0±0,1	28,0±0,2
Females	14,2±1,8	21,8±1,9	12,0±0,1	30,0±0,2

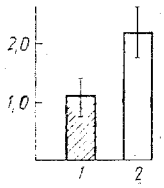

Fig. 1. Ratio of specific radioactivities of DNA of CBA males and females after injection of ^{14}C-DMH. Ordinate, ratio of specific radioactivity (in relative units). 1) Liver, 2) kidneys.

more than 24 h. DMH was isolated by the phenolic method with reprecipitation in ethanol, and treatment with protease and RNase.

EXPERIMENTAL RESULTS

The demethylating activity of microsomes isolated from the liver and kidneys of male and female CBA mice was compared, using DMH and aminopyrine (AP) as the substrates. Microsomal fractions isolated from the liver of male and female CBA mice were found not to differ significantly in their demethylating activity when DMH or AP was used as the substrate (Table 1). The velocity of demethylation of AP by liver microsomes of males and females was higher than the velocity of demethylation of DMH, i.e., the microsomal enzyme system of the liver in both male and female CBA mice exhibits higher substrate specificity for AP than for DMH.

Specificity of a different character was found when the microsomal fraction isolated from the kidneys of CBA mice was used as the metabolic system. In this case the velocity of demethylation of AP was lower than when this compound was metabolized by liver microsomes. In this case also, just as when liver microsomes were used, no difference was observed between activity of AP metabolism in males and females.

Meanwhile microsomes isolated from the kidneys of male and female mice differed significantly in their ability to metabolize DMH: the velocity of demethylation of DMH by microsomes from the female kidneys. Comparison of the demethylating activity of microsomes from the liver and kidneys of animals of the two sexes showed that a particularly high specificity for demethylation of DMH is characteristic of microsomes from the male kidney. When microsomes from male kidneys were used the velocity of metabolism of DMH was actually a little higher than that of AP, whereas enzymes of the microsomal fraction of the liver in females and males and from the kidneys of females were more highly specific for demethylation of AP.

Since the ratio between the velocities of AP and DMH metabolism by renal microsomes of male CBA mice differs considerably from that for the other three types of tissue studied it can be concluded that metabolism of these two substrates it effected by different forms of enzymes.

To study which factors may influence activity of the demethylating enzyme system, causing differences in the rate of metabolism of DMH by microsomes isolated from the kidneys of male and female CBA mice, we studied the role of sex hormones in the modification of this activity. It was found that castration of the mice reduced the rate of demethylation of DMH by male renal microsomes by half compared with the level in intact animals.

In intact CBA males, for instance, the rate of demethylation of DMH (in μmoles/30 min/mg protein) in the microsomal enzyme system of the kidneys was 24.3 ± 0.1, in castrated males 12.2 ± 0.1, and in castrated males receiving TP 23.5 ± 0.1. The level of renal demethylase activity in castrated CBA males corresponded roughly to the level of demethylase activity in intact females. These data indicate that demethylase activity of the male kidney may be regulated by the hormonal status of the animal. In this case it might be expected that administration of the hormone TP to castrated males would stimulate the demethylase activity of microsomal enzymes of the male kidney. In fact, injection of TP into castrated CBA males caused an increase in demethylase activity of the male kidney almost to its level in intact animals.

These results thus indicate that sex hormones undoubtedly affect the activity of enzyme systems participating in DMH demethylation. Similar data on the stimulating effect of androgens on metabolism of carcinogens in the kidneys were obtained in Ames' system with respect to dimethylnitrosamine (DMNA) [3]. Evidently TP induces activity of the demethylating enzymes of the kidney. It is an interesting fact that TP selectively stimulates activity of only those forms of enzymes in the male kidney which catalyze demethylation of DMH, but not of AP, since the velocities of demethylation of AP by renal microsomes of males and females, just as during metabolism of AP by liver microsomes, did not differ significantly.

Since DMH metabolism in the CBA male kidney takes place more rapidly than in females, interaction between the metabolites formed in the course of the reaction with cell targets must also take place more intensively. Accordingly an attempt was made to determine whether the higher level of DMH metabolism in the kidneys of CBA males leads to an increase in incorporation of DMH metabolites into DNA in the CBA male kidney compared with incorporation into DNA in the CBA female kidney.

Total incorporation of label into renal DNA was determined in CBA males and females. It must be pointed out that during metabolism of labeled nitroso compounds *in vivo* the appearance of radioactivity in DNA may be due not only to alkylation of the bases of this molecule by active metabolites of the carcinogen, but also to incorporation of labeled ^{3}H or ^{14}C into it in the course of nucleic acid synthesis [8]. In a proliferating tissue such as, for example, the intestine, a large part of the total label in DNA will be introduced by incorporation of labeled precursors. Against the background of this incorporation, it will be impossible to estimate the contribution of labeled alkylated bases to total activity of DNA in a proliferating tissue. However, in tissues with a sufficiently small number of dividing cells the quantity of newly synthesized DNA is small. For example, there is evidence that the quantity of ^{14}C-guanine in DNA of the resting liver 4 h after intraperitoneal injection of ^{14}C-DMNA was two orders of magnitude less than the quantity of 7-methylguanine, whereas in the stage of DNA synthesis after partial hepatectomy these values were of the same order of magnitude [10]. Thus the quantity of label entering DNA together with labeled procursors in the resting liver in the course of DNA synthesis can be disregarded, and it can be assumed that the total incorporation of label into DNA corresponds to the content of alkylated bases, mainly 7-methylguanine.

With respect to its degree of proliferation, kidney tissue is not more active than liver [5], and accordingly, when estimating incorporation of ^{14}C-DMH metabolites into renal DNA, we simply determined total incorporation of label into DNA. Absolute values of specific radioactivity of DNA isolated from the kidneys varied from one experiment to another within wide limits: from 0.6 to 3.5 cpm/μg DNA in males and from 0.3 to 1.5 cpm/μg DNA in females. In all cases, however, specific radioactivity of DNA of male kidneys exceeded the specific radioactivity of DNA of the female kidneys, whereas no such differences were found for hepatic DNA of males and females (Fig. 1).

The level of alkylation of DNA in the kidneys and liver of females and males thus corresponded to the level of metabolic activity of the microsomal enzymes isolated from these same organs, i.e., correlation was observed between these two characteristics, as was expected. The higher level of incorporation of label into renal DNA of CBA males as a whole reflects qualitatively the situation observed *in vivo* during induction of tumors of the renal capsule by DMH in CBA mice. However, quantitative correlation was not observed under these circumstances: tumors of the renal capsule do not appear in general in females, whereas the level of incorporation of a carcinogen into renal DNA of CBA females was only 50% less than the corresponding incorporation into male renal DNA. Possibly greater differences are found in the quantity of those adducts which may be related to initiation of neoplastic transforma-

tion of cells, such as ^{6}O-methylguanine, the formation of which, it has been suggested, is responsible for the carcinogenic effect of nitroso compounds, whereas when total binding of residues of the carcinogen with DNA is studied differences in the quantity of these adducts are canceled out due to the large quantity of other alkylated bases of DNA.

A definite contribution to the change in ratio between the quantity of adducts in renal DNA of males and females may be made by the DNA repair system: differences in the quantity of persistent adducts in the DNA of these two types of tissue may perhaps be increased with time due to the difference in activity of this system in the renal tissue of CBA males and females. Whatever the case, the relatively high level of DMH metabolism in the male kidneys and increased incorporation of metabolites of this carcinogen into DNA, found in the present experiments, indicate that in this tissue there is high activity of processes related to the stage of initiation of carcinogenesis, and it is evidently this which primarily determines its sensitivity to the carcinogenic action of DMH. Sex hormones may be the factors which regulate the activity of these processes in the kidneys, as our data showing the ability of TP to induce demethylase activity in the male kidney indicate.

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