Meanwhile, it must be pointed out that, according to the evidence of Rosato et al. [6], intradermal injection of malignant cells of varied histogenesis, previously treated with neuraminidase, into 25 cancer patients gave an immunotherapeutic effect.

The results now described, together with those of other investigators, suggest that neuraminidase can be used as an immunotherapeutic agent not only experimentally, but also clinically.

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EFFECT OF PRECURSORS OF ENDOGENOUSLY SYNTHESIZED CARCINOGEN DIMETHYLNITROSAMINE ON ACTIVITY OF ITS DEMETHYLASE IN RAT LIVER

E. D. Karpilovskaya and B. L. Rubenchik

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Peroral administration of dimethylamine, sodium nitrite, and a combination of these two precursors of endogenously synthesized dimethylnitrosamine to rats increases the activity of the demethylase of this carcinogen in the liver microsomes. Under chronic experimental conditions the addition of dimethylamine to the rats' diet stimulates demethylase activity even if the diet contains casein, an inducer of this enzyme system. Actinomycin D, an inhibitor of protein synthesis, prevents the increase in demethylase activity in the microsomal fraction induced by dimethylamine.

KEY WORDS: demethylase; dimethylamine; dimethylnitrosamine; sodium nitrite; endogenous synthesis; rat liver microsomes

Much attention has recently been paid to the possibility of formation of the carcinogenic agent dimethylnitrosamine (DMNA) in vivo from sodium nitrite ($NaNO_2$) and amines (or amides) [5, 6]. However, no attempt has so far been made to consider whether these precursors of endogenously synthesized DMNA have any effect on microsomal demethylase, with which the mutagenic and carcinogenic action of this compound is linked [3, 7]. The study of the factors controlling demethylase activity and synthesis in the microsomes would help to elucidate the mechanism of action of DMNA, one of the most active carcinogens and one which causes tumors in six species of laboratory animals.

The object of this investigation was to study the effect of dimethylamine (DMA), $NaNO_2$, and a combination of these two precursors of endogenously synthesized DMNA on the activity of the demethylase of this nitrosamine in the rat liver.

Laboratory of Dietary Carcinogenic Factors, Kiev Research Institute of Dietary Hygiene, Ministry of Health of the Ukrainian SSR.

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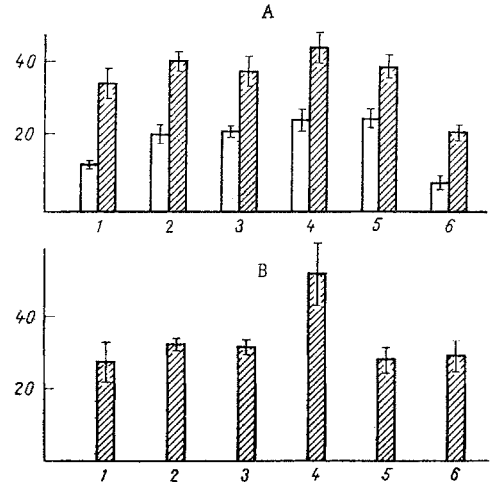

Fig. 1

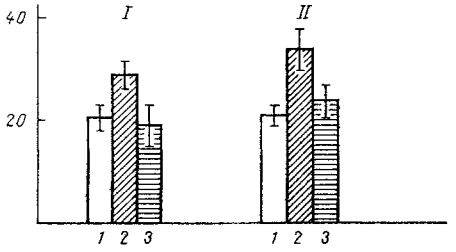

Fig. 2

Fig. 1. Activity of DMNA demethylation in liver microsomes of rats on diet with and without casein. Ordinate, activity in μmoles formaldehyde/grams microsomal protein in 30 min at 37°C. A) Acute experiments, B) chronic experiments. 1) Control; 2) DMA + $NaNO_2$; 3) DMA + $NaNO_2$ + ascorbic acid; 4) DMA; 5) $NaNO_2$; 6) DMNA. Shaded columns indicate casein present, unshaded control, casein absent.

Fig. 2. Effect of actinomycin D on increase in DMNA demethylase activity induced by DMA. I) Single, II) triple injection of DMA and actinomycin D into rats (see: Experimental Method). 1) Control; 2) DMA; 3) DMA + actinomycin D. Remainder of legend as in Fig. 1.

EXPERIMENTAL METHOD

Acute experiments were carried out on 100 young male rats weighing 140-200 g. The acute experiments were carried out on five groups of rats, which received a single dose via gastric tube of DMA (1.5 g/kg), $NaNO_2$ (125 mg/kg), DMA + $NaNO_2$ (in the same doses), DMA + $NaNO_2$ + ascorbic acid (the last in a dose of 180 mg/kg), or DMNA (15 mg/kg) respectively. Half of the animals in each group were isolated 24 h before peroral administration of the above-mentioned substances, and each rat was given 5 g of casein, which induced DMNA demethylase in the liver. The other half of the rats remained on a diet without casein.

In the chronic experiments casein was given in a daily dose of 2 g per rat as a permanent constituent of the diet. DMA (150 mg/kg), $NaNO_2$ (38 mg/kg), DMA + $NaNO_2$ (in the same doses), DMA + $NaNO_2$ + ascorbic acid (the last in a dose of 150 mg/kg), or DMNA (6 mg/kg) respectively was added to the diet of the five groups of rats. The conditions under which the rats of the control (six) groups were fed and kept were the same as the experimental, but they received no chemicals. The rats were decapitated 92 h after acute poisoning and after 3.5 months in the chronic experiments.

In two series of experiments the action of actinomycin D on DMA-induced synthesis of demethylase was studied in 30 rats. In the experiments of series I actinomycin D was injected intraperitoneally in a dose of 40 μg per rat 1 h before administration of DMA (1.5 g/kg) by gastric tube. In the experiments of series II actinomycin D was injected intraperitoneally in a dose of 10 μg per rat daily for 3 days 1 h before injection of DMA (500 mg/kg). The rats were killed 24 h after injection of the inhibitor of protein synthesis in the experiments of series I and 72 h after in series II.

Microsomes were isolated from the liver by centrifugation, in the cold, of the fraction not containing nuclei and mitochondria for 1 h at 104,000g. Demethylase activity in the microsomal fraction was determined by the method of Archakov et al. [1], using DMNA as the substrate.

EXPERIMENTAL RESULTS

It was shown previously that casein is an inducer of DMNA demethylase [8]. The results now obtained confirmed these observations. As Fig. 1A shows, in acute experiments on rats receiving casein the demethylase activity was considerably higher than in animals kept on a casein-free diet. Induction of the enzyme system by casein was found in rats of all groups ($P < 0.01$), including those poisoned with DMNA, which leads to inhibition of demethylase activity.

In the absence of casein (inducer) in the diet, poisoning the rats with DMA and $NaNO_2$, separately or together, also caused a significant (compared with the corresponding control) increase in demethylase activity in the liver ($P < 0.01$). An increase in activity could be observed by comparing the liver of the control and experimental rats receiving casein, especially in those poisoned with DMA, but the changes were not significant. However, in the chronic experiments in which rats were kept for a long time on a diet containing casein, the addition of DMA to the diet caused a significant increase in demethylase activity in the microsomes ($P < 0.05$) (Fig. 1B).

Indirect evidence of endogenous synthesis of DMNA from the precursors under these experimental conditions was given by the appearance of necrosis and necrobiosis against the background of degenerative changes in the liver and an increase in the serum glutamate–alanine transaminase activity in rats receiving a combination of DMA and $NaNO_2$. The doses of the precursors for the acute experiments were taken from the paper by Cardesa et al. [2], but the changes mentioned above in the rats were most marked after 96 h, and not after 48 h as is stated by these workers in their paper.

The level of DMNA formed was evidently very low [9] and did not lead to inhibition of demethylase activity, as did the larger dose of this carcinogen in the acute experiments. In the chronic experiments DMNA did not inhibit demethylase after 3.5 months, but after 6.5 months the activity of the enzyme was 21.3 ± 2.3 compared with 32.4 ± 3.1 in the control ($P < 0.01$).

The increase in demethylase activity compared with the control in the liver of the rats not receiving casein was due to the action of DMA and $NaNO_2$ on the enzyme system and not to DMNA formation. This was shown by the equal effect of these precursors following their combined administration to rats in the absence and in the presence of ascorbic acid, which prevents the nitroso reaction and synthesis of the nitroso-compound [6].

In the acute and even in the chronic experiments in which a diet containing the demethylase inducer casein was given, DMA showed itself capable of enhancing the activity of this enzyme system. This suggested that DMA itself is a demethylase inducer. Experiments accordingly were carried out with actinomycin D, which inhibits protein synthesis and depresses the induction of nonspecific microsomal oxidases [4]. Two series of experiments (Fig. 2) in fact showed that administration of this antibiotic to the rats prevented the increase in demethylase activity induced by DMA.

It can thus be concluded from these experiments that the intake of DMA, a precursor of endogenously synthesized DMNA, by rats may affect the metabolism of this carcinogen by increasing the activity of the system for its demethylation in the liver.

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