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PRELIMINARY COMMUNICATION

THE HEPATIC METABOLISM OF ^{15}N LABELLED DIMETHYLNITROSAMINE IN THE RAT

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SUMMARY

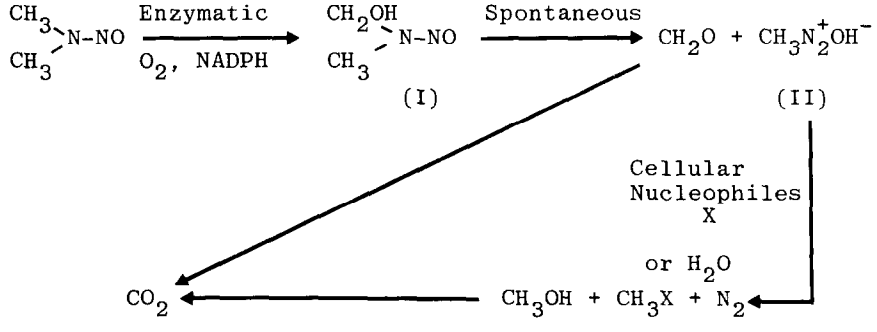
The metabolism of dimethylnitrosamine (DMN) by rat liver 10,000g supernatant fraction was found to produce gaseous nitrogen at less than 5% of the rate of either of the carbonaceous products, formaldehyde and methanol. This observation was shown to be inconsistent with the generally accepted mechanisms of nitrosamine degradation.

Dimethylnitrosamine, a potent hepatocarcinogen (1), is considered to be metabolized in rat liver initially by a cytochrome P-450 dependent pathway (2,3) involving oxidative demethylation (4,5,6) to yield formaldehyde and a methylating species. It has been suggested that the biodegradation proceeds either via an α-nitrosocarbinol intermediate (7), as shown in Scheme I, or via monomethylnitrosamine (8). The former hypothesis has received a degree of support from several studies. Lijinsky et al.(9) have shown that the methylation of guanine residues in DNA and RNA of rat liver following the administration of deuterated dimethylnitrosamine involved the transfer of an intact CD_3 group as predicted by Scheme I, but not by the alternative hypothesis in which diazomethane was the postulated alkylating agent (10). Additionally, the hydrolysis of the acetyl derivative of hydroxy methyl methyl nitrosamine (I) has been shown to produce methanol, formaldehyde and a gaseous product, presumed to be nitrogen (11).

Further support for the metabolism of dimethylnitrosamine proceeding by this route has been inferred from the observation that this acetyl derivative is mutagenic in drosophila and carcinogenic in the rat (12), although the site of tumour formation is different (13).

Earlier results from this laboratory have shown that the metabolism of dimethylnitrosamine in the intact rat and by rat liver preparations leads to the formation of methanol (14). In this paper we present our findings on nitrogen evolution on hydrolysis of the acetyl derivative of (I) and the degradation of dimethylnitrosamine by rat liver.

SCHEME I



Experimental

^{15}N labelled dimethylamine hydrochloride and sodium nitrite were purchased from Prochem, London, SW19, U.K. Acetoxy dimethylnitrosamine (11) and ^{15}N labelled dimethylnitrosamine (95% ^{15}N) (15) were synthesised by published methods. Chemical purity and isotopic purity were established by combined gas chromatography - mass spectrometry using a 5 ft. glass column containing carbowax 20 M (10%) on acid washed celite (100-120 mesh) at 120°C (14) with helium as carrier gas connected via an all glass single stage jet separator to a VG 7070F organic mass spectrometer interfaced to a VG 2040 data system. Computer calculated total ion current was used for the estimation of chemical purity and the appropriate computer calculated single ion current ratios for isotopic purity.

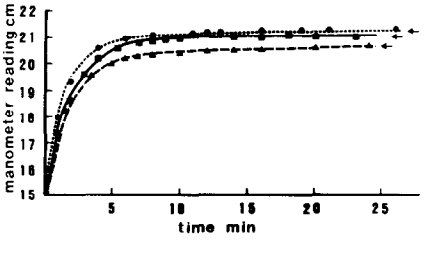
Rat liver 10,000g supernatant fractions were prepared (14) from male Sprague-Dawley rats (approx. 100g body weight) supplied by Olac (Southern) Ltd., Bicester, Oxon, U.K., and utilised immediately. Studies of the metabolism of ^{15}N labelled DMN were carried out under an oxygen atmosphere in sealed 7 ml septum vials (Pierce, Rockford, Illinois, USA). The conditions of the incubations and cofactor requirements were as described previously (14). $^{15}N_2$ formed was measured by injecting 1.0 ml gas samples from the headspace into a stainless steel reservoir provided with a capillary leak into the mass spectrometer source. The ion current at m/e 30.00 from these samples was compared with that from 1.0 ml air (which contained 12.3 ng $^{15}N_2$ per ml at atmospheric pressure and 298°K). Formaldehyde and methanol production were monitored by the Nash colorimetric technique and gas chromatography respectively as described by Lake *et al*.(14).

The non-specific esterase catalysed solvolysis of acetoxy dimethlnitrosamine was carried out as described by Roller *et al*. (11) and the N_2 released determined by Warburg manometry. In a separate experiment the gas liberated was collected and identified as pure nitrogen by mass spectrometry and the quantitative production of methanol and formaldehyde (11) was confirmed.

Results

The release of N_2 gas during the solvolysis of hydroxy dimethylnitrosamine produced by the esterase catalysed hydrolysis of acetoxy dimethylnitrosamine is shown in Fig.1. The quantity of N_2 produced was found to be 100% of the theoretical yield. The same result was obtained when acetoxy dimethylnitrosamine was incubated with esterase and rat liver 10,000g supernatant fraction in the presence of NADPH and O_2.

FIG. 1



Release of N_2 gas during solvolysis at 37°C of acetoxy dimethylnitrosamine (2.85 µmole). Tris-HCl buffer pH 7.4 (3.1 ml) containing 1 mg esterase.

Table 1 shows the production of $^{15}N_2$, methanol and formaldehyde during the metabolism of ^{15}N labelled DMN by rat liver 10,000g supernatant fractions.

TABLE 1

Product Determined	Method	Rate of Formation[a] µmole/g liver/hr.
$^{15}N_2$	MS.	0.114 ± 0.018 (9)[b]
CH_3OH	GC.	1.88 ± 0.23 (9)[b]
CH_2O	NASH	2.42 ± 0.21 (20)[b]

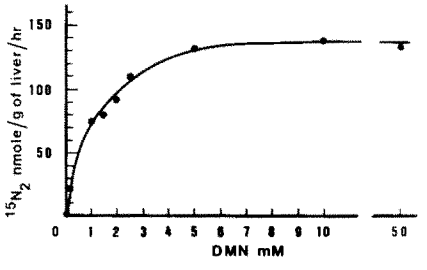
[a] Rate of product formation during $(CH_3)_2\text{-}^{15}N\text{-}^{15}NO$ (5 mM) metabolism by rat liver 10,000g supernatant fraction.

[b] Results expressed as mean ± S.E.M. (number of observations).

The rate of formation of $^{15}N_2$ was linear with time over a 60 min incubation period of a complete reaction system whereas the low blank signal obtained from an incubation system which contained all components other than either the nitrosamine or the tissue fraction did not alter with time. In the absence of NADPH or O_2 no measurable amount of $^{15}N_2$ was formed. The rate of N_2 production was not greater than 5% of that of formaldehyde.

Investigations into the variation in the rate of $^{15}N_2$ production with substrate concentration (Fig.2) showed that the maximal rate of N_2 formation was achieved at concentrations of DMN in excess of 5 mM.

FIG. 2



$^{15}N_2$ formation from $(CH_3)_2\text{-}^{15}N\text{-}^{15}NO$ by rat liver 10,000g supernatant fraction as a function of substrate concentration.

The effect on the degradation of DMN to N_2 and formaldehyde of pretreatment of the rats with sodium phenobarbitone is shown in Table 2. In the strain of rats employed sodium phenobarbitone produced a comparable increase in the rate of metabolism of DMN to the two products N_2 and formaldehyde. The rates of metabolism in the presence of two in vitro inhibitors of cytochrome P-450 dependent mixed function oxidase activities, SKF 525A and Metyrapone, are also shown in Table 2. Whereas SKF 525A slightly inhibits the formation of both of these products of DMN metabolism, Metyrapone causes an increase in metabolic rate (16). Aminoacetonitrile, a known inhibitor of DMN metabolism, toxicity and carcinogenicity (17) as well as amine oxidase activity (18) was also found to inhibit the production of N_2 (Table 2).

The rate of metabolism of DMN ^{15}N to $^{15}N_2$ and H_2CO by 10,000g supernatant fractions prepared from the livers of olive baboons was also determined and found to be similar to the rat. Again the rate of nitrogen production was found to be much less than that of formaldehyde.

TABLE 2

Pretreatment or in vitro inhibitor	Rate of $^{15}N_2$ production % Control	Rate of H_2CO production % Control
5 x 100mg/kg sodium phenobarbitone	135* (4)	140** (4)
Aminoacetonitrile (10 mM)	23***(4)	47***(4)
SKF 525A (1 mM)	55***(4)	61***(4)
Metyrapone (1 mM)	180***(3)	162***(3)

Effect of pretreatment and in vitro modulation of DMN metabolism by rat liver 10,000g supernatant. Results expressed as % control value. Number of independent experiments in brackets. P values for groups compared with controls are:

$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

DISCUSSION

According to either of the two schemes suggested (7,8) for the metabolism of dimethylnitrosamine, the degradation of this nitrosamine should lead to the formation of methanol, formaldehyde and nitrogen. Whereas the amounts of methanol and formaldehyde generated would depend on the availability of nucleophiles and the activities of enzymes involved in the metabolism of the alcohol and aldehyde, the quantity of nitrogen evolved must be directly related to the consumption of dimethylnitrosamine. Indeed, the putative intermediate, α-hydroxy methyl methylnitrosamine was found to degrade with the quantitative evolution of nitrogen.

In clear contrast, the amount of nitrogen produced during the biodegradation of dimethylnitrosamine by rat liver preparations was unexpectedly low and accounted for less than 5% of the nitrosamine decomposed. These findings were not unique to the rat; similar results were obtained with liver from baboons (unpublished observations). Thus our results are clearly at variance with the proposition that the degradation of dimethylnitrosamine proceeds by the intermediacy of either the α-nitrosocarbinol or monomethylnitrosamine derivative. At best these routes would constitute a very minor component of the pathway(s) involved in the mammalian metabolism of this nitrosamine.

The results of this study taken in conjunction with our earlier findings questioning the assumption that the cytochrome P-450/448 dependent mixed function oxidase system is the sole mediator of dimethylnitrosamine metabolism, suggest that an alternative explanation for the relevant mechanisms involved must be sought. Clearly, a crucial issue is the metabolic fate of the nitrogen moiety, and studies directed to providing an answer to this question are currently in progress.

Acknowledgements

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