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MUTAGENICITY OF ALKYL-(ω-HYDROXYALKYL) NITROSAMINES RELATED TO DIBUTYLNITROSAMINE

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Summary

Various alkyl-(ω-hydroxyalkyl) derivatives related to dibutylnitrosamine (DBN) were investigated for mutagenicity in the absence of liver-activation system. Butyl-(4-hydroxybutyl)-, butyl-(3-hydroxypropyl)-, and butyl-(2-hydroxyethyl)-nitrosamines were so tested and found to be mutagenic for TA 1535 strain of *Salmonella typhimurium*. In all cases, a simple dose—response relationship was observed. Furthermore, no significant ($p < 0.05$) differences in the mutagenicity of the various test compounds were observed as the alkyl side-chain possessing the OH group increased in length. From these results it is suggested that mutagenesis in *S. typhimurium* by the higher dialkylnitrosamines is partially due to the formation of ω-hydroxylated derivatives in addition to the major mutagenic metabolite derived from α-carbon dealkylation.

Introduction

Studies on dialkylnitrosamine-induced mutagenesis in various indicator organisms have utilized DMN, a relatively well-understood mutagen and carcinogen [8,11,14,22]. Recently, the need for a better understanding of nitrosamine-induced mutagenesis has shifted emphasis toward the study of a large array of nitrosamines and their metabolites [9,12,15,18,20,21]. The

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Abbreviations: BBN, butyl-(4-hydroxybutyl)nitrosamine; BHBN-3, butyl-(3-hydroxybutyl)-nitrosamine; BHCPN, butyl-(2-hydroxy-3-carboxypropyl)nitrosamine; BHEN, butyl-(2-hydroxyethyl)-nitrosamine; BHPN, butyl-(3-hydroxypropyl)nitrosamine; BOPN, *N*-butyl-*N*-(2-oxopropyl)nitrosamine; DBN, dibutylnitrosamine; DMN, dimethylnitrosamine; HEHBN, *N*-(2-hydroxyethyl)-*N*-(4-hydroxybutyl)nitrosamine; NEE, *N*-nitroso-2-(ethylamino)ethanol.

mutagenicity of the higher members of the homologous series such as DBN is thought to be attributable to product(s) of oxidative dealkylation at the carbon adjacent to the amine-nitrogen Druckery et al. [5,6]. The α-hydroxylation hypothesis explains reasonably well the mutagenicity of all dialkylnitrosamines, regardless of the number of carbon atoms in the alkyl chain. The α-position is only one of a number of sites available for oxidation, other sites being β, ω, ω-1 [3,4,10,16,19,20]. Although these derivatives are not necessarily the "active" or "proximal" mutagens, they nevertheless represent oxidation products which may be mutagenic in the absence or presence of a metabolic activation system.

BBN, BHPN, and BHEN were compared for mutagenic activity using *S. typhimurium* strain (TA 1535) [1]. The selection of alkyl-(ω-hydroxyalkyl)-nitrosamines used in this study was based on the following considerations: BBN is a known metabolite of DBN [3,4] and recent work by Okada and Hashimoto [17] on the carcinogenic nature of certain alkyl-(ω-hydroxyalkyl)nitrosamines shows that not all terminally-hydroxylated dialkylnitrosamines are carcinogenic. Studies [2,7] demonstrating the carcinogenic nature of alkyl-(ω-hydroxyalkyl)nitrosamines has led to the assumption that all ω-hydroxylated nitrosamines are carcinogenic. However, Okada and Hashimoto [17] have shown that BHPN is non-carcinogenic to rats whereas the other ω-hydroxylated nitrosamines studied were carcinogenic. It was felt therefore that in vitro microbial mutagenesis studies would help resolve the findings regarding the biological effects of ω-hydroxylated nitrosamines, since many compounds that are carcinogenic have also been shown to be mutagenic.

Materials and methods

Synthesis of ω-hydroxylated N-nitroso compounds

The alkyl-(ω-hydroxyalkyl)nitrosamines (BBN, BHPN and BHEN) were synthesized from commercially available starting materials according to the procedure of Druckery et al. [6]. Nitrosation of the secondary amine resulted in a mixture of alcohol [alkyl-(ω-hydroxyalkyl)nitrosamine] and nitrite ester. Extensive acid hydrolysis was required to convert the nitrite ester to the alcohol. Distillation in vacuo produced a pale, yellow, viscous oil: BBN, b.p. 127—129°C/0.09 mm Hg (Lit. 116°C/0.01 mm Hg); BHPN, b.p. 123—127°C/0.9 mm Hg (Lit. 144°C/3.0 mm Hg); and BHEN, b.p. 115—117°C/1.5 mm Hg (Lit. 148°C/7.0 mm Hg). Identity and purity of the alkyl-(ω-hydroxyalkyl)-nitrosamines were confirmed by IR spectroscopy, GC-mass spectroscopy, and thin-layer chromatography.

Mutagenesis assay

The histidine auxotroph (his^-) of *S. typhimurium* (strain TA 1535) was obtained from Dr. Bruce Ames (Biochemistry Department, University of California Berkeley, Calif.). Starter cultures of *S. typhimurium* were initiated by inoculating nutrient broth (Difco) from stock stab-cultures. When the subcultured *S. typhimurium* had increased in density to a Mac Farland No. 2 standard (1×10^8 cells/ml), the cell culture was centrifuged at 1500 rpm for 15 min and resuspended in sterile 0.85% saline. Plates were prepared according to the pro-

cedure described by Ames and co-workers [1]. To 2.0 ml top agar containing approximately 1×10^7 bacteria. 20 µl aliquot of test compound (100 µg, 250 µg, 500 µg and 1000 µg) in dimethyl sulfoxide was added. Revertant colonies were scored after incubation at 37°C for 48 h. Control plates, lacking the mutagen, were prepared for detecting spontaneous (his^+) reversions.

Results

BBN and related compounds, namely BHEN and BHPN, were shown to be mutagenic in the *absence* of a metabolic activation system. Revertant colonies (his^- to his^+) were scored after incubation at 37°C for two days. The induced mutation frequencies at varying doses of alkyl(ω-hydroxyalkyl)nitrosamines are shown in Fig. 1 and Table 1. Values for mutation frequencies were corrected by subtracting control cultures lacking test compound from cultures exposed to the test agent (control 8.5 ± 0.5 colony forming units/plate). Increased concentrations of the ω-hydroxylated nitrosamines resulted in elevated mutation frequencies when plotted as the number of colonies versus µg of compound (Fig. 1). The mutagenic activities of the various alkyl-(ω-hydroxyalkyl) compounds, differing from one another in the length of the side-chain containing the OH group, were not statistically significant as determined by Duncan's multiple-range test ($p < 0.05$).

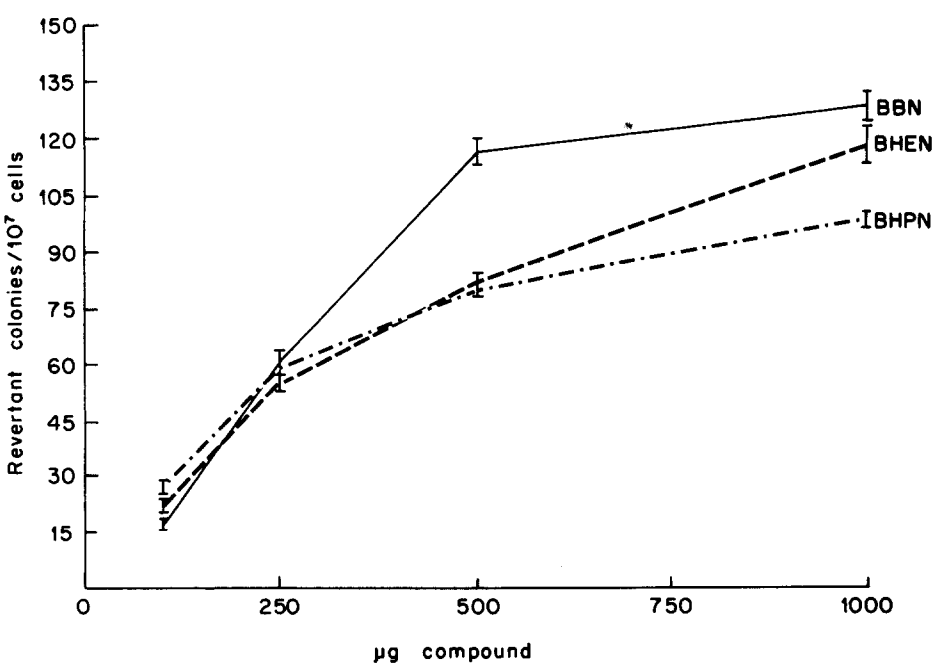

Fig. 1. Dose—effect relationships for the mutagenic activity induced by alkyl-(ω-hydroxyalkyl)nitrosamines in *S. typhimurium* (Strain TA 1535).

TABLE 1

MUTATION FREQUENCIES IN HISTIDINE AUXOTROPHS OF *S. TYPHIMURIUM* (STRAIN TA 1535) INDUCED BY ALKYL-(ω-HYDROXYALKYL)NITROSAMINES AT VARYING CONCENTRATIONS OF TEST COMPOUNDS

N-Nitrosamine	Dose (μg)	Revertant colonies/plate
BBN	100	17.6 ± 2.3
BBN	250	61.2 ± 4.1
BBN	500	116.9 ± 7.7
BBN	1000	130.0 ± 8.5
BHPN	100	27.6 ± 3.3
BHPN	250	60.8 ± 4.4
BHPN	500	80.4 ± 2.4
BHPN	1000	98.3 ± 3.1
BHEN	100	22.8 ± 1.8
BHEN	250	55.4 ± 4.9
BHEN	500	83.0 ± 2.5
BHEN	1000	120.0 ± 11.0
Control		8.5 ± 0.5

Control values were subtracted from the number of colonies counted for ω-hydroxylated nitrosamine-treated bacteria.
Results are expressed as mean ± S.E.

Discussion

In the absence of liver microsomes, BBN, BHPN and BHEN were found to be mutagenic for *S. typhimurium* (TA 1535). These studies suggest that oxidation products of the higher dialkylnitrosamines are mutagenic in the absence of a metabolic activation system. In contrast to our findings, Okada et al. [18] and Nagao et al. [13] have shown BBN to be non-mutagenic for *S. typhimurium* strain (TA 1535) in the absence of liver extract. Hsieh et al. [9] have examined NEE, a ω-hydroxylated nitrosamine, for mutagenic activity and found this compound to be non-mutagenic for *S. typhimurium* (TA 1535). In the study by Nagao and co-workers [13], mutagenesis studies of selected hydroxy and oxo derivatives of DBN showed the following compounds to be mutagenic in the absence of liver homogenate: BHBN-3, BHCPN, and BOPN. Furthermore, various di-(ω-hydroxyalkyl)nitrosamines, namely, HEHBN and related compounds were definitely but weakly mutagenic in the absence of an activation system.

To explain the apparent discrepancy between our results with mono-(ω-hydroxyalkyl)nitrosamines and those of Okada et al. [18] and Nagao et al. [13], we have focused on the quantity and availability of mutagen to the bacteria. In our procedure, the possibility of a non-specific interaction between the test compounds and the growth media was eliminated by the utilization of resuspended bacteria in 0.85% saline. The findings of Nagao et al. [13] concerning the mutagenicity of various hydroxylated derivatives would seem to indicate that any or all hydroxylated derivatives of a higher dialkylnitrosamine, e.g. DBN, are potentially mutagenic in the absence of a metabolic activation system. Furthermore, it is evident that there is an absolute requirement of the

microsomal fraction for the conversion of parent compound to the hydroxylated moiety, a metabolic conversion capability which is apparently lacking in bacteria. However, bacteria (as shown by our data and by Nagao [13]) can convert the hydroxylated derivatives to mutagenic products. The mutagenicity of the various hydroxylated nitrosamines reflects a combined mutagenic effect from the product(s) derived from $\alpha,\beta,\omega,\omega$-1 hydroxylation. Additional work is needed to determine those oxidation products of higher dialkylnitrosamines which are mutagenic per se or require further metabolism to exert their mutagenic effect.

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