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METABOLIC ACTIVATION OF NITROSAMINES TO MUTAGENS BY VARIOUS ANIMAL SPECIES INCLUDING MAN

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INTRODUCTION

The nitrosamines comprise a ubiquitous group of potent chemical carcinogens which, surprisingly, when incorporated into the Salmonella/microsome mutagenicity test may either exhibit a very weak mutagenic response or no mutagenicity at all (1,2). The nitrosamines are indirect-acting carcinogens requiring metabolic activation and it is conceivable that their poor mutagenic response is the result of the rat being an inappropriate animal species to use as the source of the activation system. Marked species differences in the bioactivation of carcinogens to mutagens in the Ames test has already been reported for aromatic amines where the hamster, in contrast to the rat, was a very efficient activator (3). In the present paper we report the bioactivation of five nitrosamines to mutagens by various animal species including man.

METHODS

Male golden Syrian hamsters (100-120 g), male Wistar albino rats (150-200 g) and male CD1 mice were used. The human liver sample was from a male, a traffic accident victim, and fresh pig liver was obtained from the local abattoir, both samples being stored at -80^{o}C. Hepatic post-mitochondrial supernatant (S9 fraction) and microsomal suspension were prepared and the following determinations were carried out in the post-mitochrondrial supernatant: kynuramine oxidase (4), dimethylaniline N-oxidase (5) and benzphetamine N-demethylase (6); in the microsomal suspension, ethoxyresorufin O-deethylase (7), NADPH-cytochrome c reductase (8) and cytochrome P-450 (9); protein was determined in both fractions (10). Efficiency of activation to mutagens was determined using the Ames test and employing Salmonella typhimurium strain TA 100 (11). Double the usual amount of S9 fraction was used in the S9 mix, i.e. 100 µl per plate, and each nitrosamine at five concentrations (250-2000 µg/plate) was preincubated with the activation system and bacteria for 30 minutes at 37^{o}C.

RESULTS AND DISCUSSION

Three enzyme systems have been implicated in the bioactivation of nitrosamines; the cytrochrome P-450 dependent mixed-function oxidases, the mixed-function amine oxidase and monoamine oxidase. Not all three

enzyme systems contribute to the activation of all nitrosamines. For example the cytochrome P-450 enzyme system appears not to participate in the activation of dimethylnitrosamine to mutagens (12,13), which may be catalysed by a microsomal monoamine oxidase (14). Neither of these enzyme systems is involved in the bioactivation of nitrosomorpholine (15). All activation systems employed in the present study were fully characterised with respect to these enzyme systems (Table 1).

TABLE 1 CHARACTERISATION OF ACTIVATION SYSTEMS USED IN THE AMES TEST

	Mouse	Hamster	Rat	Pig	Human
4-Hydroxylation of kynuramine (nmol/min per 100 µl of S9 fraction)	0.15	0.16	0.26	0.58	0.54
N-oxidation of dimethylaniline (pmol/min per 100 µl of S9 fraction)	41.8	44.8	27.1	47.5	12.3
N-Demethylation of benzphetamine (nmol/min per 100 µl of S9 fraction)	6.41	11.50	4.25	2.99	4.94
O-Deethylation of ethoxyresorufin (pmol/min per 100 µl of S9 fraction)	20	53	40	17	16
Cytochrome P-450 (nmol/100 µl of S9 fraction)	0.46	0.69	0.44	0.18	0.14
Protein (mg/100 µl of S9 fraction)	2.2	2.0	2.2	3.1	2.7

TABLE 2 BIOACTIVATION OF NITROSAMINES TO MUTAGENS BY VARIOUS ANIMAL SPECIES AND MAN

Results are presented as the average of triplicate plates from which the spontaneous reversion rate (44-75) has been subtracted. Triplicates did not differ from each other by more than 15%. Results for only one carcinogen concentration are shown.

Carcinogen	Concentration (µg/plate)	Histidine Revertants/plate				
		Mouse	Hamster	Rat	Pig	Human
Dimethylnitrosamine	1000	70	123	19	13	16
Methylethylnitrosamine	1000	69	151	47	31	10
Dipropylnitrosamine	250	53	101	32	14	36
Nitrosopiperidine	1000	1033	1706	80	126	219
Nitrosopyrrolidine	1000	64	1329	8	152	1257

The simplest analogue dimethylnitrosamine was activated by the hamster with the mouse also exhibiting a doubling of the spontaneous reversion rate (Table 2); none of the other activation systems produced a positive mutagenic response. Essentially the same picture emerges with the other two aliphatic nitrosamines, the methylethyl-and dipropyl-analogues. The cyclic nitrosamine nitrosopiperidine was metabolically converted to mutagens by all activation systems, but with markedly different efficiencies, the hamster being once again the most efficient followed by the mouse (Table 2). All animal species, with the exception of the rat, could bioactivate nitrosopyrrolidine, the hamster and human preparations being by far the most effective (Table 2). No correlation was evident between the efficiency of activation of the nitrosamines by the various species and any of the enzyme systems studied suggesting that either these are not involved in the activation process or do not catalyse the rate-limiting step.

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