Bioactivation of the heterocyclic aromatic amine 2-amino-3-methyl-9H-pyrido[2,3-b]indole (MeA\textalpha C) in recombinant test systems expressing human xenobiotic-metabolizing enzymes

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2-Amino-3-methyl-9H-pyrido[2,3-b]indole (MeA\textalpha C) and some metabolites were investigated for mutagenicity in mammalian cell lines and bacterial strains engineered for the expression of human enzymes. MeA\textalpha C induced gene mutations (studied at the hpr locus) in Chinese hamster V79-derived cells co-expressing cytochrome (CYP) 1A2 and sulphotransferase (SULT) 1A1 even at a concentration of 30 nM, but was inactive in cells co-expressing CYP1A2 and N-acetyltransferase (NAT) 1 or 2. MeA\textalpha C, tested in the presence of rat liver post-mitochondrial fraction, showed strongly enhanced mutagenicity in a Salmonella typhimurium strain expressing human SULT1A1 compared with the control (recipient) strain TA1538/1,8-DNP (deficient in endogenous acetyltransferase). Mutagenicity was also enhanced, although to a lesser extent, when NAT2 was expressed in the latter strain. The metabolite, 2-hydroxy-3-amino-3-methyl-9H-pyrido[2,3-b]indole (N-OH-MeA\textalpha C) was a direct mutagen to strains TA1538 and TA1538/1,8-DNP. This mutagenicity was strongly enhanced in corresponding strains expressing SULT1A1. A moderate enhancement was observed when SULT1A2, SULT1B1, SULT1C2 or NAT2 were expressed in strain TA1538. The remaining enzymes studied (SULT1A3, 1C1, 1E1, 2A1, 2B1a, 2B1b, 4A1 and NAT1) did not indicate any activation of N-OH-MeA\textalpha C. Preliminary mutagenicity experiments in SULT-expressing S. typhimurium strains were conducted with other hydroxylated metabolites of MeA\textalpha C. The phenols, 6- and 7-hydroxy-MeA\textalpha C, were inactive under the conditions studied. The benzylic alcohol, 2-amino-3-hydroxymethyl-9H-pyrido[2,3-b]indole, was mutagenic in a strain expressing SULT1A1, but its activity was much weaker than that of N-OH-MeA\textalpha C. Thus, N-hydroxylation (e.g. mediated by CYP1A2) and sulpho conjugation (primarily mediated by SULT1A1) was the dominating activation pathway of MeA\textalpha C in model systems engineered for human enzymes. Some other SULT forms as well as NAT2 were also capable of activating N-OH-MeA\textalpha C, although with much lower efficiency than SULT1A1. Another minor activation pathway involved benzylic hydroxylation followed by sulpho conjugation by SULT1A1.

Introduction

Heterocyclic aromatic amines are formed during the heating of food containing free amino acids and other small nitrogen-containing molecules, such as creatinine in muscle meat (1–3). Many heterocyclic amines have demonstrated carcinogenic activity in animal models (2–4). Mutagenicity appears to be a major mechanism of their carcinogenicity (5–7). Heterocyclic amines are not mutagenic as such, but can be metabolized to highly reactive intermediates that form DNA adducts (8–10). Although the xenobiotic-metabolizing system is conserved among vertebrates in principle, critical species-dependent differences can occur in traits such as the substrate specificity, tissue distribution and other regulation processes of specific enzymes (11–13). These differences are a major factor leading to species-dependent carcinogenicity and organotropisms of carcinogens. As many of these enzymes are genetically polymorphic and/or are induced by dietary habits in humans, they also may affect the individual susceptibility. For these reasons, it is interesting to identify the enzymes involved in the activation of a given compound. During the last years, we have expressed a large number of human and other mammalian xenobiotic-metabolizing enzymes in target cells of standard mutagenicity tests, such as Ames’s his\textsuperscript{-}S. typhimurium strains and Chinese hamster V79 cells. In the present study we have used these systems to specify the activation pathways of 2-amino-3-methyl-9H-pyrido[2,3-b]indole (MeA\textalpha C) (structural formula in Figure 1).

MeA\textalpha C is an abundant heterocyclic amine. It has been found in cooked food, such as meat, chicken and fish (14–17), in cigarette smoke condensate (18–21) and in wine (22). It appears to be formed primarily as a pyrolysis product of tryptophan. Dietary administration of MeA\textalpha C to CDF1 mice led to the formation of hepatic and vascular tumours (23). In the rat, this treatment showed weak initiation and strong promotion of enzyme-altered (pre-neoplastic) foci in liver (24) and it produced hepatocellular carcinomas, fibrosarcomas in subcutis, pancreatic acinar cell adenomas as well as severe renal cytotoxicity (25). MeA\textalpha C induced morphological transformation in the C3H/M2 mouse fibroblasts cell line (26). It demonstrated mutagenic effects to bacteria, but was much less potent than some other heterocyclic amines, such as 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) (5,26–28). Using 3P-postlabelling analysis, two major DNA adducts were detected in MeA\textalpha C-treated hepatocytes from rats; the dominating adduct appeared to be N2-deoxyguanosin-8-yl-MeA\textalpha C (29). A total of five DNA adducts were detected in pancreatic and hepatic tissue of MeA\textalpha C-treated rats (30). In general, aromatic amines are bioactivated in two steps, N-oxidation by a cytochrome P450 (CYP), usually CYP1A2, followed by a conjugation (usually acetylation or sulphonation) (Figure 1). These conjugation reactions introduce good leaving groups, resulting in the formation of a highly reactive resonance-stabilized nitrenium/carbonium ion (31). Two N-acetyltransferases that

Abbreviations: CYP, cytochrome P450; DNP, Salmonella typhimurium strain TA1538/1,8-DNP (deficient in endogenous acetyltransferase); MeA\textalpha C, 2-amino-3-methyl-9H-pyrido[2,3-b]indole; NAT, acetyltransferase; PhHP, 2-amino-1-methyl-6-phenyl-9H-pyrido[2,3-b]indole; SULT, sulphotransferase.

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acetylate aromatic amines (NATs) (12,32) and 11 sulphotransferases that conjugate small molecules (SULTs) (12,32) have been detected in the human.

In the present study, we found that the major activation pathway of MeAαC requires CYP and SULT activities. CYPs do not only form 2-hydroxylamino-3-methyl-9H-pyrido[2,3-b]indole (N-OH-MeAαC) from MeAαC, but also additional metabolites that are potential substrates of SULTs. In particular, a benzylic alcohol as well as phenolic metabolites have been detected (33). Various benzylic alcohols are metabolized to reactive sulphuric acid esters (34); thus this pathway is another possible activation mechanism of MeAαC. Numerous phenols are substrates for SULTs (35), but formation of a reactive metabolite via sulphonation of a phenolic hydroxyl group has not yet been observed. Nevertheless, we have investigated the two major phenolic MeAαC metabolites (33) in addition to N-OH-MeAαC and the benzylic alcohol for mutagenicity in target cells expressing human SULTs.

Fig. 1. Metabolic pathways, discussed in the present study, of MeAαC in mammalian systems.
Materials and methods

**Chemicals**

MeAoC was obtained from Toronto Research Chemicals (Toronto, Canada). N-OH-MeAoC was synthesised as described previously (33), 6-Hydroxy-2-amino-3-methyl-9H-pyrido[2,3-b]indole (6-OH-MeAoC), 7-hydroxy-2-amino-3-methyl-9H-pyrido[2,3-b]indole (7-OH-MeAoC) and 2-amino-3-hydroxymethyl-9H-pyrido[2,3-b]indole (3-OHCH2-Aoc) were obtained by incubating MeAoC with hepatic microsomal preparations and subsequent purification by high-performance liquid chromatography (33). The purity of the N-OH-MeAoC exceeded 90%, the major impurity was the parent compound. The purity of the 6-OH-MeAoC, 7-OH-MeAoC and 3-OHCH2-Aoc was better than 95% as determined by high-performance liquid chromatography with UV detection.

**Mutagenicity studies in bacteria**

_Salmonella typhimurium_ strains TA1538 and TA100 were generously provided by Dr B.N.Ames (Berkeley, CA). Strain TA1538/1.8-DNP (designation shortened to DNP in the present paper), which lacks an endogenous acetyltransferase (36), was a kind gift of Dr D.Wild (Bundesanstalt für Fleischforschung, Kulmbach, Germany). Human SULTs and NATs were stably expressed in these strains as described previously (37,38). The designations of these strains are composed of the recipient strain and the expressed human enzyme.

The purified enzyme protein was used as a standard. The expression levels could be estimated in some strains by immunoblotting. SULT1A1 constitutes 0.7-1.4% of the cytosolic protein of strains TA1538-SULT1A1 (37) and DNP-SULT1A1 (unpublished result). This level is nearly five times that observed in a liver sample studied concurrently. As estimated by immunoblotting, levels of SULT1A2 were 2-4% of the cytosolic protein in strain TA1538-SULT1A2 IZ (37) and 0.7-1.4% of that in strain DNP-SULT1A2 (unpublished result). We have only detected SULT1A2 immunoreactive protein in relatively few liver samples. In all cases, this level was much lower than that of SULT1A1.

No purified standards were available for most other SULTs. However, after electrophoresis of cytosolic preparations on polyacrylamide gels under denaturing conditions and Coomassie blue staining, an additional protein band (which also reacted with the appropriate anti-SULT antibody) was detected in each recombinant strain. We estimate that the intensity of the additional band varied from 6-fold weaker to 3-fold stronger compared with SULT1A1 in strain TA1538-SULT1A1.

Using an antibody raised against NAT1, strong signals were detected in cytosolic preparations of strains DNP-NAT1 and DNP-NAT2 (38). The intensity of the immunosignals was similar in these strains, but the electrophoretic mobility of the expressed NAT forms differed. Corresponding bands were also detected in eight cytosolic liver samples studied concurrently. However, all cytosolic preparations of strains DNP-NAT1 and DNP-NAT2 (38). The intensities of the cytosolic protein of strains TA1538-SULT1A1 (37) and DNP-SULT1A1 were 2\(\times\)10\(^{-4}\) of that in strain DNP-SULT1A2 (unpublished result). We have only detected SULT1A2 immunoreactive protein in relatively few liver samples. In all cases, this level was much lower than that of SULT1A1.

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Mutagenicity was determined using a modified version of the assay described by Maron and Ames (39). A detailed protocol of the modification used for the direct test has been described elsewhere (37). The experiments using a hepatic metabolizing system were conducted as follows. Liver postmitochondrial (S9) fraction was prepared from Acoro helix 1254-treated male rats as described previously (40). The incubation mixture comprised 100 mM of 50% mix (0.1 mg S9 protein, 1.9 mg bovine serum albumin, 8 mM MgCl\(_2\), 20 mM MgSO\(_4\), 83 mM KCl, 4 mM NADP, 5 mM glucose 6-phosphate and 53 mM sodium phosphate buffer, pH 7.4), 100\(\mu\)l of the bacterial suspension (0.5-1 x 10\(^8\) cells) and the test compound (in 10\(\mu\)l dimethylsulphoxide). After incubation for 60 (in the direct test) or 40 min (in the experiments using S9 mix) at 37°C, 2.0 ml of 45°C warm soft agar (5.5 mg/ml agar, 5.5 mg/ml NaCl, 15 mg/ml biotin, 50 \(\mu\)M histidine, 25 mM sodium phosphate buffer, pH 7.4) was added, and the mixture was poured onto a Petri dish containing 24 ml minimal agar (15 mg/ml agar in Vogel-Bonner E medium with 20 mg/ml glucose). After incubation for 3 (DNP-derived strains) or 2 days (other strains) in the dark, the colonies (his\(^+\) revertants) were counted. Incubations were carried out in triplicate. Specific mutagenicities (revertants per nmol) were calculated from the slope of the initial part of the dose–response curve.

**Mutagenicity studies in Chinese hamster V79 cells**

A V79-derived cell line that stably expresses human CYP1A2 was a generous gift of Prof. J.Dochner (GenPharmTox Biotech AG, Martinsried, Germany). The original designation of this cell line is XEMh1-A2-M2' (41). It is termed here V79-CYP1A2. Human NAT1, NAT2 and SULT1A1 were expressed additionally in this cell line. Details concerning the construction and characterization of these new cell lines will be published elsewhere (E.Muckel, U.Pabel and H.R.Glatt, manuscript in preparation). Briefly, the cDNAs for wild-type forms of human NATs (NAT1', NAT2'4) and human SULT1A1'1 in plasmid pPK223-3 and that of human SULT1A1'1 in plasmid pPK223-2 were used for cloning into the eukaryotic expression vectors pSfI (Promega, Mannheim, Germany) and pPSPV (42), respectively. The cDNA constructs were introduced into V79-CYP1A2 cells using a co-transfection strategy with a vector conferring resistance to puromycin (43). The level of CYP1A2 protein in V79 cells expressing NATs and SULTs was similar to that observed in the recipient cell line V79-CYP1A2 (41), as demonstrated by immunoblotting of microsomal fractions and the mutagenic response of \((\pm)\)-benz(a)pyrene-trans-7,8-dihydrodiol in the hprt gene mutation assay (data not shown). The levels of immunoreactive SULT1A1, NAT1 and NAT2 protein in the recombinant V79 cell lines were 10-20% of the figures for the corresponding recombinant _Salmonella_ strains. Therefore, the level of SULT1A1 in V79-CYP1A2-SULT1A1 cells was in the high physiological (hepatic) range, whereas levels of NATs were low. Details concerning the construction and changes in the recombinant strains. Therefore, the level of SULT1A1 in V79-CYP1A2-SULT1A1 cells was in the high physiological (hepatic) range, whereas levels of NATs were low. Details concerning the construction and changes in the recombinant strains. Therefore, the level of SULT1A1 in V79-CYP1A2-SULT1A1 cells was in the high physiological (hepatic) range, whereas levels of NATs were low. Details concerning the construction and changes in the recombinant strains. Therefore, the level of SULT1A1 in V79-CYP1A2-SULT1A1 cells was in the high physiological (hepatic) range, whereas levels of NATs were low. Details concerning the construction and changes in the recombinant strains. Therefore, the level of SULT1A1 in V79-CYP1A2-SULT1A1 cells was in the high physiological (hepatic) range, whereas levels of NATs were low. Details concerning the construction and changes in the recombinant strains. Therefore, the level of SULT1A1 in V79-CYP1A2-SULT1A1 cells was in the high physiological (hepatic) range, whereas levels of NATs were low. Details concerning the construction and changes in the recombinant strains. Therefore, the level of SULT1A1 in V79-CYP1A2-SULT1A1 cells was in the high physiological (hepatic) range, whereas levels of NATs were low. Details concerning the construction and changes in the recombinant strains. Therefore, the level of SULT1A1 in V79-CYP1A2-SULT1A1 cells was in the high physiological (hepatic) range, whereas levels of NATs were low. Details concerning the construction and changes in the recombinant strains. Therefore, the level of SULT1A1 in V79-CYP1A2-SULT1A1 cells was in the high physiological (hepatic) range, whereas levels of NATs were low. Details concerning the construction and changes in the recombinant strains. Therefore, the level of SULT1A1 in V79-CYP1A2-SULT1A1 cells was in the high physiological (hepatic) range, whereas levels of NATs were low. Details concerning the construction and changes in the recombinant strains. Therefore, the level of...
mutagenic to V79-CYP1A2-NAT1 and V79-CYP1A2-NAT2 cells, but not to V79-CYP1A2 cells, demonstrating that NATs were active under the condition of the mutagenicity assay (data not shown). Likewise, the CYP-dependent positive control, trans-benzo[a]pyrene-7,8-dihydrodiol, induced similar mutagenic effects to cell lines V79-CYP1A2, V79-CYP1A2-SULT1A1, V79-CYP1A2-NAT1 and V79-CYP1A2-NAT2, but was inactive to the parental cell line V79 (data not shown).

Mutagenicity of MeA\textsubscript{a}C to bacterial target cells
MeA\textsubscript{a}C was weakly mutagenic to strains DNP and DNP-NAT1, and substantially more active to DNP-NAT2 and DNP-SULT1A1 when tested in the presence of rat liver S9 mix (Figure 3, summarized results in Table I). In the absence of S9 mix, it was inactive in all these strains. These results imply that enzymes present in S9 mix as well as specific enzymes expressed in the recombinant strains were involved in the activation of MeA\textsubscript{a}C. S9 mix primarily provides CYP/monooxygenation activity. Among the human enzymes expressed in the bacteria, SULT1A1 showed the strongest enhancement of the S9-mediated mutagenicity of MeA\textsubscript{a}C, followed by NAT2, whereas NAT1 did not show any effect.

In subsequent experiments, we studied the mutagenicity of hydroxylated metabolites of MeA\textsubscript{a}C and used strains expressing all human SULTs and NATs identified.

Mutagenicity of N-OH-MeA\textsubscript{a}C to bacterial target cells
N-OH-MeA\textsubscript{a}C was mutagenic to strain DNP even in the absence of liver S9 mix (Figure 4, Table I). Its mutagenicity was higher by a factor of 1.3 (what is within the experimental variation) in strain DNP-NAT1, a factor of 2.9 in strain DNP-NAT2, a factor of 32 in strain DNP-SULT1A2 and a factor of 68 in strain DNP-SULT1A1. Thus, two human SULTs were substantially more effective in the activation of N-OH-MeA\textsubscript{a}C than were human NATs. Other human SULTs have not yet been expressed in DNP. However, they have been

**Fig. 3.** Mutagenicity of MeA\textsubscript{a}C in the presence of rat liver S9 to *S. typhimurium* DNP-derived strains engineered for the expression of various human enzymes. Values are means and SE of three plates.

**Fig. 4.** Mutagenicity of N-OH-MeA\textsubscript{a}C to *S. typhimurium* DNP-derived strains engineered for the expression of various human enzymes. Values are means and SE of three plates.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Induced revertants per nmol*</th>
</tr>
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<tbody>
<tr>
<td>DNP</td>
<td>10</td>
</tr>
<tr>
<td>DNP-NAT1</td>
<td>7</td>
</tr>
<tr>
<td>DNP-NAT2</td>
<td>450</td>
</tr>
<tr>
<td>DNP-SULT1A1</td>
<td>3100</td>
</tr>
<tr>
<td>DNP-SULT1A2</td>
<td>–</td>
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<tr>
<td>TA1538</td>
<td>–</td>
</tr>
<tr>
<td>TA1538-SULT1A1</td>
<td>–</td>
</tr>
<tr>
<td>TA1538-SULT1A2c</td>
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</tr>
<tr>
<td>TA1538-SULT1A3</td>
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<td>TA1538-SULT1C2</td>
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<tr>
<td>TA1538-SULT2B1b</td>
<td>–</td>
</tr>
<tr>
<td>TA1538-SULT4A1</td>
<td>–</td>
</tr>
<tr>
<td>TA100-SULT1A1</td>
<td>–</td>
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</tbody>
</table>

*aInitial slope of the dose-mutagenicity curve. Some curves are presented in Figures 3–6. For negative results, an estimate of the limit of detection is given (number of spontaneous revertants divided by highest dose used). –, not tested.

*bValues obtained in the presence of rat liver S9 mix. No mutagenicity was obtained in the same strains when S9 mix was omitted (detection limit <1 revertants/nmol).

*cThe high-expression strain TA1538-SULT1A2*1Z (37) was used.

*dMedian of four separate experiments (whose individual values were 6000–22 000 revertants/nmol).
expressed in TA1538, which differs from DNP by the presence of an endogenous acetyltransferase. Strain TA1538 was somewhat more responsive (4-fold) to the mutagenic action of N-OH-MeAOC than was strain DNP (Table I), suggesting some activation by its endogenous acetyltransferase (OAT). However, we had observed similar differences between these strains with an ultimate mutagen, benzo[a]pyrene 4,5-oxide, that does not contain any possible acceptor group for acetylation. Moreover, plates with DNP-derived strains required a longer incubation time until revertant colonies appeared than those with OAT-proficient TA1538-derived strains, indicating a slower growth rate. Thus, the moderate difference in the responsiveness between strains TA1538 and DNP may have unspecific reasons and does not conclusively demonstrate an activation of N-OH-MeAOC by OAT.

In the series of TA1538-derived strains (Figure 5, Table I), expression of human SULT1A1 strongly potentiated the mutagenicity of N-OH-MeAOC, as it had done in the series of DNP-derived strains. Expression of human SULT1A2, 1B1 and 1C2 also enhanced the mutagenicity of N-OH-MeAOC compared with strain TA1538, although to a weaker extent than observed with strain TA1538-SULT1A1. The mutagenic effects observed in the other strains (expressing SULT1A3, 1C1, 1E1, 2A1, 2B1a, 2B1b or 4A1) were within the inter-experimental variation of the effect observed in the parental TA1538 (Figure 5, Table I), suggesting that N-OH-MeAOC is a poor substrate, if any, for these SULT forms.

**Mutagenicity of C-hydroxylated metabolites of MeAOC to bacterial target cells**

Several C-hydroxylated metabolites were produced by incubating MeAOC with hepatic microsomal systems and then purified by high-performance liquid chromatography (33). Therefore, they were available in small quantities and could only be tested for mutagenicity under a few selected conditions and at low dose levels. The phenols, 6- and 7-OH-MeAOC, were inactive under the conditions used (Figure 6, Table I). Specifically, they were at least 1800 and 5500 times less active than TA1538-SULT1A1 at N-OH-MeAOC. The benzyl alcohol, 3-OHCH2-AOC, was tested in strains that had shown strong mutagenic responses towards some other benzyl alcohol (34). 3-OHCH2-AOC was inactive in strains TA1538-SULT1E1 and TA1538-SULT2A1, but enhanced the number of revertants with strains TA1538-SULT1A1 and TA100-SULT1A1 (Figure 6, Table I). The mutagenic activity of 3-OHCH2-AOC to strain TA1538-SULT1A1 was 17 000 times lower than that of N-OH-MeAOC (Table I). The latter compound has not yet been investigated with TA100-SULT1A1.

**Discussion**

Other investigators had found that MeAOC is a bacterial mutagen but only in the presence of an external metabolic activating system (5,26-28). This finding was confirmed in the present study. In addition, we demonstrated for the first time that MeAOC is also mutagenic to mammalian cells, again requiring the presence of activating enzymes.

The mutagenicity of MeAOC was in need of an oxidizing enzyme (liver S9 mix or cDNA-expressed CYP1A2). In mammalian cells, a conjugating enzyme was absolutely necessary for a positive test result; in bacterial test systems, it strongly enhanced the mutagenicity of MeAOC and the investigated metabolites. In addition, we studied four hydroxylated metabolites of MeAOC for mutagenicity in bacteria.

In all five model systems leading to positive test results (MeAOC/V79 cells, MeAOC/DNP-derived strains, N-OH-MeAOC/DNP-derived strains, N-OH MeAOC/TA1538-derived strains and 3-OHCH2-AOC/TA1538-derived strains), human SULT1A1 was the most efficient conjugating enzyme investigated leading to activation. Among the other 10 human SULTs examined, SULT1A2, 1B1 and 1C2 also demonstrated activation of N-OH-MeAOC (in TA1538-derived strains), although at a substantially lower level than SULT1A1.

Among the human NATs investigated, NAT1 did not indicate any activation in the three models investigated (MeAOC/V79 cells, MeAOC/DNP-derived strains, N-OH-MeAOC/DNP-derived strains), whereas the results with NAT2 depended on the system used: no influence in the MeAOC/V79 cell system (using human CYP1A2 for the first activation step), a moderate enhancement of the mutagenicity of N-OH-MeAOC in the DNP-derived strain (2.9-fold) and a relatively strong enhancement of the S9-mix-dependent mutagenicity of MeAOC (45-fold) [although less than by SULT1A1 (310-fold)] in DNP-derived strains. Thus, it is possible that metabolites other than N-OH-MeAOC (e.g. additionally containing a C-hydroxyl group) are formed by rat liver enzymes and are more efficiently activated by NAT2 than is N-OH-MeAOC. Alternatively, the different results may be due...
to different steady state levels and/or different competing enzymes in the various systems used.

We have shown in a previous study that the level of the expressed enzyme may affect the bioactivation of various promutagens and the resulting mutagenic effect (37). We have not studied the influence of the expression level in the present study. However, the most effective conjugating enzyme, SULT1A1, was expressed in the V79 system at a level that is in the high physiological (hepatic) range and at a nearly 5-fold higher level in *Salmonella*. The expression of the other SULT forms in *Salmonella* varied from 1/6 to 3 times the level of SULT1A1 in strain TA1538-SULT1A1, but none of these forms reaches a comparably high level with SULT1A1 in human liver. The expression levels of the NATs expressed in V79 cells and *Salmonella* strains were very high compared with their natural expression levels in human liver (see description of the test systems in the Materials and methods section). These findings corroborate the view that SULT1A1 may be the dominating conjugating enzyme in the activation of MeA-C in the human, although other enzymes could also be important in specific cells.

Functional genetic polymorphisms have been detected for three conjugating enzymes involved in the activation of MeA-C, NAT2 (reviewed in ref. 32), SULT1A1 and SULT1A2 (47–50). Marked differences in the activation of various promutagens have been observed between cDNA-expressed alloenzymes of SULT1A1 and SULT1A2 (37,51). It will be interesting to see whether this is also true with promutagenic metabolites of MeA-C and whether polymorphisms affect the individual susceptibility towards this compound.

Various, but not all, heterocyclic amines demonstrated enhanced mutagenicity to a DNP-derived *Salmonella* strain engineered for the expression of NAT2 compared with control strain DNP (52). Likewise, we observed that the mutagenicity of 2-hydroxylamino-3-methylimidazo[4,5-f]quinoline was strongly enhanced when NAT2 was expressed in strain DNP, but unaffected when SULT1A1 was expressed (38). However, 2-hydroxylamino-1-methyl-6-phenylimidazo[4,5-b]-pyridine showed the reverse response, a strongly enhanced mutagenicity to strain DNP-SULT1A1 and an unaffected activity to strain DNP-NAT2 compared with strain DNP (38). Thus, different heterocyclic amines require different conjugating enzymes for their activation. The activation of MeA-C in the human appears to be similar to that of PhIP, with a dominating role of SULTs, in particular SULT1A1. It is probable that the kind of conjugating enzyme involved in the activation of a mutagen/carcinogen is important for its organotropisms, as each enzyme has its own tissue distribution and the resulting ultimate carcinogens are short-lived and, therefore, may cause more damage in the cells in which they are generated than elsewhere. Human, rat and murine SULT1A1 are highly expressed in liver. In line with these findings, the liver was a major target tissue for the carcinogenicity of MeA-C in mice (23) and rats (25). However, it is important to note that the tissue distribution of SULTs strongly varies between species. In the adult rat, most forms, including SULT1A1, are chiefly restricted to the liver (12). The mouse has not been sufficiently investigated in this regard. Various human SULTs show high expression in specific extrahepatic tissues and some of them are absent in liver (12). Although human SULT1A1 is particularly high in liver, it is also detected at substantial levels in numerous extrahepatic tissues. Therefore, not only the liver but also many other tissues may be potential target tissues for carcinogenicity by MeA-C in the human.

The usual activation pathway of aromatic amines involves N-hydroxylation followed by a conjugation reaction. This mechanism was confirmed in the present study for MeA-C. In addition, we observed a second activation mechanism: the benzylic alcohol 3-OHCH2-A-C was mutagenic in SULT1A1-expressing bacteria, but was much less potent than N-OH-MeA-C in the same system. This difference in potency may be biased as 3-OHCH2-A-C and the inactive metabolites 6-OH-MeA-C and 7-OH-MeA-C were only available in very small quantities and therefore could be tested only under a few selected conditions. It is probable that activated N-OH-MeA-C and 3-OHCH2-A-C form different DNA adducts in the target cells used. It will be interesting to see which of these adducts are formed in tissues of MeA-C-treated animals and of humans exposed to heterocyclic amines.

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