GLUCURONIDATION OF THE LUNG CARCINOGEN 4-(METHYLNITROSAMINO)-1-(3-PYRIDYL)-1-BUTANOL (NNAL) BY RAT UDP-GLUCURONOSYLTRANSFERASE 2B1

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ABSTRACT:

4-(Methylnitrosamo)-1-(3-pyridyl)-1-butanone and its major metabolite, 4-(methylnitrosamo)-1-(3-pyridyl)-1-butanol (NNAL), are potent lung carcinogens in animals. UDP-glucuronosyltransferase (UGT)-mediated glucuronidation of NNAL is a potentially important detoxification pathway for these carcinogens. To identify the UGT isozyme(s) involved in this pathway, we examined the glucuronidation of NNAL in rat liver microsomes and homogenates from cell lines overexpressing specific UGT isozymes. NNAL glucuronidation was induced in liver microsomes from rats treated with family 2 UGT inducers including phenobarbital and 3,5-di-tert-butyl-4-hydroxytoluene, which exhibited 1.7- and 2.6-fold higher rates of glucuronidation than microsomes from control rats. The rates of NNAL glucuronidation in liver microsomes from Gunn (deficient in family 1 UGTs) and RHA parental control rats were similar. All rat liver microsomes used in the present study catalyzed the glucuronidation of (S)-NNAL at a rate between 3.5 and 5.5 times that of the glucuronidation of (R)-NNAL. Liver microsomes from Wistar rats exhibiting the low-androsterone glucuronidation phenotype characteristic of the UGT2B2-deficient genotype glucuronidated NNAL at a rate similar to microsomes from Wistar rats exhibiting the high-androsterone glucuronidation phenotype/UGT2B2 (+) genotype. Homogenates from UGT2B1-overexpressing cells catalyzed a major metabolite of NNAL-Gluc I was the major diastereomer formed by UGT2B1. Glucuronidation of NNAL was not detected with homogenates from UGT2B12-overexpressing cells. These results suggest that UGT2B1 plays an important role in the glucuronidation of NNAL in the rat.

One of the most abundant potent procarcinogens in tobacco and tobacco smoke is the nicotine-derived tobacco-specific nitrosamine, 4-(methylnitrosamo)-1-(3-pyridyl)-1-butanol (NNK) (Hecht, 1998b). NNK induces predominantly lung adenocarcinomas in rodents independent of the route of administration (reviewed in Hecht, 1998). In the Fischer 344 rat, NNK induces pancreatic tumors (Rivenson et al., 1988) and oral cancer (Carmella et al., 1993; Hecht et al., 1993; Hecht, 1998), with an estimated 39 to 100% of the NNK dose converted to NNAL glucuronides (NNAL-Gluc; see Fig. 1A). NNK reduction to NNAL occurs in rodents, monkeys, and humans (Carmella et al., 1993; Hecht et al., 1993; Hecht, 1998), with an estimated 39 to 100% of the NNK dose converted to NNAL in smokers (Carmella et al., 1998). NNAL is activated via pathways similar to those observed for NNK and, like NNK, is a potent lung and pancreatic carcinogen in rodents (Rivenson et al., 1988; Hecht, 1998). In a reaction likely to be catalyzed by the UDP-glucuronosyltransferase (UGT) family of enzymes, NNAL is also metabolized to its glucuronide, β-O-[4-(methylnitrosamo)-1-(3-pyridyl)-1-but-1-yl]-D-glucosiduronic acid (NNAL-Gluc; see Fig. 1A); Morse et al., 1990; Carmella et al., 1993; Hecht et al., 1993; Hecht, 1998). Because NNK is metabolized to both the (S) and (R) enantiomers of NNAL, two diastereomeric glucuronides of NNAL are formed (Fig. 1B). These have previously been referred to as NNAL-Gluc I, formed from (S)-NNAL, and NNAL-Gluc II, formed from (R)-NNAL (Hecht et al., 1997). NNK-treated rats form predominantly NNAL-Gluc I (Hecht et al., 1997; Murphy et al., 1997) due primarily to the preferential glucuronidation of (S)-NNAL rather than...
preferential reduction of NNK to (∈)-NNK. In a recent study, rat liver microsomes were shown to metabolize racemic NNK to NNAL-Gluc I (Staretz et al., 1997). Therefore, in the rat, the predominance of NNAL-Gluc I appears to be due to the preferential glucuronidation of (∈)-NNK.

Both NNAL and NNAL-Gluc are excreted in the bile of rats (Shulze et al., 1992) and in the urine of animals, smokers, and individuals exposed to sidestream smoke (Morse et al., 1990; Hecht et al., 1994; Murphy et al., 1994; Carmella et al., 1995; Richie et al., 1997; Parsons et al., 1998). Although the formation of NNAL is clearly not a detoxification pathway for NNK, the glucuronidation of NNAL appears to be an important mechanism for NNK detoxification. Recent studies have demonstrated that, in contrast to the high tumorigenicity exhibited by both (∈)- and (∈)-NNAL, NNAL-Gluc is nontumorigenic after s.c. injection into A/J mice (Upadhyaya et al., 1999). In addition, the glucuronidation of (∈)-NNAL was significantly greater than (∈)-NNAL after injection into A/J mice, a pattern consistent with the higher tumorigenicity exhibited by (∈)-NNAL in the same experiments (Upadhyaya et al., 1999). Kim and Wells (1996) also reported that skin fibroblasts from UGT family 1-deficient rats were more sensitive to NNK-mediated cytotoxicity. Significant interindividual variability in the ratio of NNAL-Gluc:NNAL excreted in human urine has been observed, suggesting that individuals may differ greatly in their ability to detoxify NNK (Carmella et al., 1995). It has been hypothesized that the ability of an individual to glucuronidate NNAL may directly affect their susceptibility to lung and potentially other tobacco-related cancers.

UGTs are a superfamily of enzymes that glucuronidates many xenobiotics and endogenous compounds (Tephly and Burchell, 1990). Based on differences in sequence homology and substrate specificity, two families of UGTs (UGT1 and UGT2) have been identified in several species, each containing several highly homologous UGT genes. We have shown previously that the human family 1 isozyme UGT1A9 (for updated nomenclature, see Mackenzie et al., 1997) catalyzed the glucuronidation of NNAL; no activity was observed with the related human UGT family 1 isozyme UGT1A6 (Ren et al., 1996). In the rat, phenobarbital (PB), which has been associated with the induction of family 2 UGT isozymes in the rat (Mackenzie, 1986; Pritchard et al., 1994; Emi et al., 1995; Green et al., 1995), induces the glucuronidation of two NNK metabolites, 4-(hydroxymethylamino)-1-(3-pyridyl)-1-butanoate and 4-hydroxy-1-(3-pyridyl)-1-butanoate (Murphy et al., 1994, 1997). The goal of the present study was to elucidate the UGT(s) responsible for the glucuronidation of NNAL. We demonstrate that the NNAL glucuronidation pathway is specific to UGT family 2 isozymes in rats and that one of the major isoforms involved in this metabolic pathway is UGT2B1.

Materials and Methods

Chemicals and Materials. PB, 3-methylcholanthrene (MC), clofibrate acid, 2,3,4,5-tetrahydropyridine-4-carboxylate (BHA), 3,5-di-tert-butyl-4-hydroxytoluene (BHT), 4-nitrophenol (4-NP), androsterone, UDP-glucuronic acid (UDPGA), 1,2-lysophosphatidyl choline palmital C16:0, and β-glucuronidase were purchased from Sigma (St. Louis, MO). Bilirubin was obtained from Fluka Chemicals (Ronkonkoma, NY). [14C]UDPGA (specific activity = 318 Ci/mol) was obtained from ICN Pharmaceuticals (Costa Mesa, CA), and [3H]NNK (specific activity = 2.4 Ci/mmol) as well as unlabeled NNK were purchased from Chemsyn Scientific (Lenexa, KS). Dulbecco’s modified Eagle’s medium was obtained from Mediatech (Herndon, VA), whereas fetal bovine serum and geneticin were purchased from GIBCO BRL Life Technologies (Grand Island, NY). Internal standards for NNAL glucuronidation studies, including 4-hydroxy-1-(3-pyridyl)-1-butanoate (HPB), 4-(methylnitrosoamino)-1-(N′-oxo-3-pyridyl)-1-butanoate (NNK-N-oxide), 4-(methylnitrosoamino)-1-(N′-oxo-3-pyridyl)-1-butanol (NNAL-N-oxide), 4-hydroxy-1-(3-pyridyl)-1-butanol (diol), and 4-oxo-4-(3-pyridyl)-butyric acid (keto acid), were kind gifts from Shantu Amin at the American Health Foundation (Valhalla, NY).

Animal Studies. Sprague-Dawley (SD) rats (150–200 g, female), GUNN rats (j/j; 150–200 g, female), and RHA rats (+/+; 150–200 g, female) were purchased from Harlan Sprague-Dawley, Inc. (Indianapolis, IN). SD rats (n = 3–5 per group) were treated with one of the following: PB (40 mg/kg body weight × 1 day⁻¹ for 4 days), clofibrate acid (250 mg/kg body weight × 1 day⁻¹ for 3 days), 3-MC (40 mg/kg body weight × 1 day⁻¹ for 4 days) or vehicle (corn oil or water) by i.p. injection and fed rat chow (NIH07) ad libitum. GUNN and

![Fig. 1. Simplified schematic of NNK metabolism to NNAL-Gluc (A) and structures of NNAL and NNAL-Gluc rotamers and enantiomers (B).](image-url)
The precipitation was further analyzed using the BCA assay as described above or by thin-layer chromatography (TLC) and autoradiography (Bansal and Gessner, 1980; described below). [14C]Glucuronidated products were analyzed by radioflow HPLC as described above or by thin-layer chromatography (TLC) and autoradiography (Bansal and Gessner, 1980; described below). 14C[NNAL-gluc peaks were tentatively identified by relative retention time, then confirmed by sensitivity to treatment with E. coli β-glucuronidase at 37°C.

### Glucuronidation Assays

**Ugt activity toward 4-NP in rat liver microsomes was determined using the method of Burchell and Weatherill (1981).** Enzyme reactions (125 µl final volume) containing 250 mM Tris-HCl (pH 7.5), 5 mM MgCl2, 4 mM UDPGA, 5 µg of di-β-l-2-lysophosphatidyl choline palmital C16:0, 25 µg of protein, and 0.5 mM 4-NP were incubated at 37°C for 5 min. The rate of 4-nitrophenol-O-glucuronide (4-NP-gluc) formation was linear for up to 10 min for all rat liver microsomal samples tested. Reactions were terminated by the addition of 2 vol of 0.5 M ice-cold trichloroacetic acid and centrifuged at 17,000 g in a microcentrifuge for 5 min. The supernatants were collected and analyzed by spectrophotometry at 400 nm for loss of absorbance to determine the conversion of 4-NP to its glucuronide. The presence of 4-NP-gluc was confirmed by sensitivity to treatment by E. coli β-glucuronidase as described above.

#### Glucuronidation in rat liver microsomes

**Table 1**

<table>
<thead>
<tr>
<th>Rat species</th>
<th>Inducer</th>
<th>Rate of NNAL-Gluc Formation</th>
<th>Rate of NNAL-Gluc I: NNAL-Gluc II</th>
<th>Rate of Aglycone-gluc Formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD</td>
<td>Corn oil (n = 3)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.94 ± 0.32</td>
<td>—</td>
<td>4.65 ± 0.48</td>
</tr>
<tr>
<td></td>
<td>3-MC (n = 5)</td>
<td>1.10 ± 0.21</td>
<td>1.2</td>
<td>4.58 ± 0.41</td>
</tr>
<tr>
<td></td>
<td>H2O (n = 5)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.01 ± 0.28</td>
<td>—</td>
<td>4.25 ± 0.70</td>
</tr>
<tr>
<td></td>
<td>PB (n = 4)</td>
<td>1.74 ± 0.39</td>
<td>1.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.80 ± 0.49</td>
</tr>
<tr>
<td></td>
<td>Clofibric acid (n = 5)</td>
<td>1.41 ± 0.30</td>
<td>1.4</td>
<td>4.37 ± 0.60</td>
</tr>
<tr>
<td>Wistar</td>
<td>None (n = 5)</td>
<td>0.46 ± 0.08</td>
<td>—</td>
<td>3.46 ± 1.10</td>
</tr>
<tr>
<td></td>
<td>BHA (n = 5)</td>
<td>0.78 ± 0.30</td>
<td>1.7&lt;sup&gt;e&lt;/sup&gt;</td>
<td>4.03 ± 0.59</td>
</tr>
<tr>
<td></td>
<td>BHT (n = 5)</td>
<td>1.24 ± 0.49</td>
<td>2.6&lt;sup&gt;h&lt;/sup&gt;</td>
<td>5.35 ± 1.10&lt;sup&gt;j&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

* a) Data are expressed as mean ± S.D. for triplicate assays, performed at 37°C for 40 min using 2 mM [14C]UDPGA (0.5 µCi/reaction) as described in Materials and Methods.

### Statistical Analysis

The Student’s t test (two-tailed) was used for all comparative analyses. The correlation coefficient (r<sup>2</sup>) from linear regression analysis of Lineweaver-Burk plots was used to determine the K<sub>m</sub> and V<sub>max</sub> of UGT2B1 glucuronidation of NNAL.
Results

NNAL Glucuronidation in Liver Microsomes from Induced Rats. Rat UGT isozymes are responsive to various enzyme inducers (Emi et al., 1995). For example, clofibrate acid and 3-MC were previously shown to be effective inducers of the bilirubin- and phenol-metabolizing family 1 UGTs, respectively (Emi et al., 1995). BHT and PB have been previously associated with the induction of family 2 UGTs (Mackenzie, 1986; Pritchard et al., 1994; Emi et al., 1995; Green et al., 1995; Yang et al., 1996), whereas BHA was shown to induce the glucuronidation of small, planar phenols (Kashiﬁ et al., 1994; Yang et al., 1996). To examine whether the glucuronidation of NNAL is susceptible to these various inducers, the rate of NNAL glucuronidation was determined in liver microsomes prepared from untreated as well as PB-, 3-MC-, or clofibrate acid-treated SD rats. In addition, the levels of glucuronidated NNAL were examined in liver microsomes prepared previously from untreated as well as BHA- or BHT-treated Wistar rats (Kashiﬁ et al., 1994). In vitro assays demonstrated that significant levels of induction of NNAL glucuronidation were observed in liver microsomes from PB- (p < .05), BHA- (p < .05), and BHT- (p < .005) treated rats as compared to liver microsomes from corresponding untreated controls (Table 1). Significant levels of induction were not observed in liver microsomes from either 3-MC- (p = .40) or clofibrate acid- (p = .10) treated rats. A similar pattern was observed with PB, 3-MC, and clofibrate acid in Fischer 344 rats (results not shown). This pattern of induction is consistent with the possibility that NNAL glucuronidation is preferentially mediated by family 2 UGT isozymes.

4-NP is glucuronidated by multiple UGT family 1 and family 2 isozymes (Tephly and Burchell, 1990). As a control for the induction of UGT activity, the rate of 4-NP-gluc formation was shown to be significantly induced in liver microsomes from 3-MC- (p < .05), PB- (p < .05), and BHT- (p < .005) treated rats as compared to liver microsomes from PB- or BHT-treated Wistar rats. In vitro assays demonstrated that significant levels of induction of NNAL glucuronidation were observed in liver microsomes from PB- (p < .05), BHA- (p < .05), and BHT- (p < .005) treated rats as compared to liver microsomes from PB- or BHT-treated Wistar rats as well as control Wistar rats. As shown in Table 2, the rate of NNAL glucuronidation in liver microsomes from untreated control and induced SD rats was not significantly different and averaged approximately 4.5:1 (Table 1). A similar ratio was observed in liver microsomes from PB-treated rats; details in Materials and Methods.

NNAL Glucuronidation in Liver Microsomes from GUNN Rats. GUNN (j/j) rats are deficient in family 1 UGTs due to the presence of a homozygous 1-bp deletion within the common region of the UGT family 1 locus (i.e., position +1239; Iyanagi, 1991). Therefore, to analyze the role of family 1 UGTs in the glucuronidation of NNAL, the rate of NNAL glucuronidation in liver microsomes from GUNN rats was determined and compared to the rate observed in liver microsomes from parental RHA (+/+) controls. As shown in Table 2, the rate of NNAL glucuronidation was similar for liver microsomes from both strains of rat. In contrast, the rate of 4-NP glucuronidation, which is catalyzed by the phenol-metabolizing family 1 UGTs as well as

![HPLC analysis of NNAL-Gluc formation by liver microsomes from untreated and PB-treated SD rats.](dmd.aspetjournals.org)
as other UGT isozymes (Tephly and Burchell, 1990; Ikushiro et al., 1995), was approximately 3-fold lower in liver microsomes from GUNN (j/j) rats (p < .025) as compared with RHA (+/+) controls (Table 2). Therefore, the family 1 UGTs, which are deficient in the GUNN rat, do not appear to play a role in the glucuronidation of NNAL. These data are consistent with the lack of significant induction of NNAL glucuronidation observed in liver microsomes from rats treated with either 3-MC or clofibrac acid, agents that induce mainly family 1 UGTs (Ikushiro et al., 1995).

**NNAL Glucuronidation in Liver Microsomes from Untreated Wistar Rats.** Our results with inducers and the GUNN rat suggest that NNAL glucuronidation may be mediated by family 2 UGTs. Thirty-five percent of Wistar rats exhibit a homozgyous gene deletion of the family 2 isozyme, UGT2B2 (Corser et al., 1987; Homma et al., 1992). Androsterone is largely glucuronidated by UGT2B2, and this corresponds with an observed bimodal pattern of androsterone glucuronidation in Wistar rats [i.e., UGT2B2-deleted rats exhibit a low androsterone-glucuronidating phenotype (Matsui et al., 1979; Green et al., 1985; Corser et al., 1987; Homma et al., 1992)]. Therefore, to investigate the role of UGT2B2 in NNAL glucuronidation, we compared the relative rates of NNAL glucuronidation to androsterone glucuronidation in liver microsomes from untreated Wistar rats. Microsomes from three of five (60%) untreated Wistar rats examined in this study exhibited the low androsterone-glucuronidating phenotype typical of UGT2B2-deficient rats (Table 3). However, no correlation was observed between the levels of glucuronidated NNAL and androsterone phenotype. The same lack of correlation between NNAL glucuronidation and androsterone glucuronidation was also observed in liver microsomes from BHA- (n = 5) and BHT- (n = 5) treated Wistar rats (results not shown). These data suggest that UGT2B2 does not play a major role in the glucuronidation of NNAL.

**NNAL Glucuronidation in Cell Homogenates from UGT Family 2 Isozyme-Overexpressing Cell Lines.** To further investigate the role of family 2 UGTs in the glucuronidation of NNAL, we examined the rate of NNAL glucuronidation by cellular homogenates prepared from HK293 cells that overexpressed either UGT2B1 or UGT2B12 (Green et al., 1995; King et al., 1997). The NNAL glucuronidation activities of the two UGT-overexpressing cell lines was determined and compared with the activity with previously characterized substrates (Table 4). The relative glucuronidation activity of UGT2B1 was determined using clofibrac acid as the aglycone, whereas 4-NP was the aglycone used to assay for UGT2B12 activity. The glucuronidated conjugates of both aglycones were sensitive to β-glucuronidase treatment. Using these two substrates, the glucuronidation activity of homogenates prepared from UGT2B1-overexpressing cells was approximately 15.5-fold that of homogenates prepared from UGT2B12-overexpressing cells (Table 4). Therefore, to analyze presence of NNAL glucuronidating activity in the two cell lines, 15.5 times more homogenate protein was used for UGT2B12-overexpressing cells (i.e., 3.9 mg) than for UGT2B1-overexpressing cells (0.25 mg). No glucuronidation of NNAL was observed by homogenates from cells overexpressing UGT2B12 (Fig. 3D), but significant levels of NNAL glucuronidation were observed in homogenates prepared from UGT2B1-overexpressing cells (Fig. 3B; Table 4). The 14C-labeled product formed by homogenates from UGT2B1-overexpressing cells was sensitive to β-glucuronidase treatment (Fig. 3C). Based on their relative retention times when compared to: 1) the retention times of peaks 1 and 2 observed in rat liver microsomes (31–34 min; see Fig. 2) and 2) the retention times of NNAL glucuronides (Fig. 3C) of the glucuronide of (S)-NNAL (NNAL-Gluc I). The glucuronidation activity of UGT2B1 was 745 μM with a V\textsubscript{max} of 27.5 pmol/mg protein−1·min−1 as determined by Lineweaver-Burk kinetic analysis (Fig. 4). The r\textsuperscript{2} value from this analysis (r = 0.987) suggests that the enzyme kinetics of this reaction is linear at the range of NNAL concentrations used (0.25–2 mM).
In this study, we present results of experiments designed to determine the UGT isozyme(s) that play a role in the glucuronidation of NNAL in the rat. This is the first study to directly measure the glucuronidation of this potent lung carcinogen. Significant increases in the rate of NNAL glucuronidation were observed in liver microsomes prepared from rats treated with inducers of UGT family 2 isozymes. In contrast, liver microsomes prepared from rats treated with family 1 inducers did not catalyze NNAL glucuronidation at a higher rate than did noninduced microsomes. These data are consistent with the fact that no alteration in the rate of NNAL glucuronidation were observed in liver microsomes from rats deficient in UGT family 1 isozymes (i.e., in GUNN rats). Taken together, these data suggest that family 1 UGTs do not play a major role in the glucuronidation of NNAL. The fact that Kim and Wells (1996) demonstrated increased NNK-mediated cytotoxicity in skin fibroblasts from GUNN as compared to RHA control rats would suggest that, potentially, one or more UGT family 1 isozymes may be involved in the glucuronidation of other carcinogenically active NNK metabolites other than NNAL, such as 4-(hydroxymethylnitrosamino)-1-(3-pyridyl)-1-butanone. The high degree of specificity exhibited by UGT isozymes that appears to be exhibited toward NNK metabolites suggests that, potentially, multiple UGT isozymes from both UGT families may be important determinants in overall susceptibility to NNK-induced carcinogenicity.

The data presented in this study also provide information as to the NNAL-glucuronidating capacity of three family 2 rat UGT isozymes, UGT2B1, UGT2B2, and UGT2B12. Of these, only UGT2B1 appears to metabolize NNAL. The fact that homogenates of cell lines that overexpress UGT2B1 catalyze NNAL glucuronidation efficiently is consistent with the induction of NNAL glucuronidation observed in liver microsomes from rats treated with PB or BHT, because both agents have been associated with the induction of this UGT isozyme (Mackenzie, 1986; Pritchard et al., 1994; Green et al., 1995; Yang et al., 1996). Additional support for the role of UGT2B1 in the metabolism of NNAL is provided by the stereoselectivity of this reaction. NNAL-Gluc I was the major NNAL-Gluc diastereomer formed in rat liver microsomes and in homogenates from UGT2B1-overexpressing cells. In addition, BHT, which has been previously demonstrated to be a strong inducer of UGT2B1 (Yang et al., 1996), exhibited a preferential and significant induction of NNAL-Gluc I.

UGT2B1 has a very wide substrate specificity and catalyzes the glucuronidation of acids such as clofibric acid, as well as phenols such as morphine. The $K_m$ of NNAL glucuronidation by UGT2B1 (745 $\mu$M) was intermediate to other substrates studied, where the $K_m$ ranged from 12 $\mu$M for clofibric acid to 3.18 mM for morphine (Pritchard et al., 1994) and was similar to the $K_m$ of 432 $\mu$M reported for nalorephine (King et al., 1997). UGT2B1 has also been shown to...
possess a high degree of stereoselectivity. Pritchard and coworkers (1994) previously reported that UGT2B1 preferentially glucuroni-
dated the (S)-enantiomer of ibuprofen, analogous to the preference we report here for (S)-NNAL. Because relatively high levels of UGT2B1 expression were previously observed in rat liver (Macken-
zie, 1987), these data suggest that UGT2B1 plays a major role in the glucuronidation and elimination of NNAL in the rat.

The major diastereomeric form of glucuronidated NNAL in urine from snuff users (Murphy et al., 1994) as well as in microsomes prepared from human livers (Ren et al., 1996) is NNAL-Glcu II. As described in the present study, the major diastereomeric form of NNAL-Glc rat liver microsomes is NNAL-Glc I, a pattern consistent with that observed previously in urine from NNK-treated rats (Hecht et al., 1993). Although the present studies demonstrate a lack of significant activity exhibited by family 1 UGTs for NNAL in the rat, preliminary results from this laboratory demonstrated that the human family 1 UGT isozyme, UGT1A9, catalyzes the glucuronida-
tion of NNAL (Ren et al., 1996). Thus, there appears to be species-
specific differences in terms of the UGT isozymes involved in the glucuronidation of NNAL in rats as compared with humans. UGT2B1 exhibits approximately 75% homology at the nucleotide level and 60% homology at the amino acid level with several human UGTs, with the highest homology existing with UGT2B15 (77% homology at the nucleotide level, 66% at the amino acid level; analysis performed by Blast search of National Center for Biotechnology Information sequence database, National Institutes of Health). Further studies examining known human UGT isozymes in the glucuronida-
tion of NNAL are currently under way.

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