METABOLISM OF MELATONIN BY HUMAN CYTOCHROMES P450

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Received September 16, 2004; accepted December 22, 2004

ABSTRACT:

In humans, the pineal hormone melatonin (MEL) is principally metabolized to 6-hydroxymelatonin (6-HMEL), which is further conjugated with sulfate and excreted in urine. MEL O-demethylation represents a minor reaction. The exact role of individual human cytochromes P450 (P450s) in these pathways has not been established. We used a panel of 11 recombinant human P450 isozymes to investigate for the first time the 6-hydroxylation and O-demethylation of MEL. CYP1A1, CYP1A2, and CYP1B1 all 6-hydroxylated MEL, with CYP2C9 playing a minor role. These reactions were NADPH-dependent. CYP2C9 and, to some extent CYP1A2, O-demethylated MEL. The K_m (µM) and V_max (µmol min^-1 pmol^-1) for 6-hydroxylation were estimated as 19.2 ± 2.01 and 6.46 ± 0.22 (CYP1A1), 25.9 ± 2.47 and 10.6 ± 0.32 (CYP1A2), and 30.9 ± 3.76 and 53.1 ± 0.21 (CYP1B1). These findings confirm the suggestion of others that CYP1A2 is probably the foremost hepatic P450 in the 6-hydroxylation of MEL and a single report that CYP1A1 is also able to mediate this reaction. However, this is the first time that CYP1B1 has been shown to 6-hydroxylate MEL. The IC_50 for the CYP1B1-selective inhibitor (E)-2,4,3',5'-tetramethoxy stilbene was estimated to be 30 nM for MEL 6-hydroxylation by recombinant human CYP1B1. Comparison of brain homogenates from wild-type and cyp1b1-null mice revealed that MEL 6-hydroxylation was clearly mediated to a significant degree by CYP1B1. CYP1B1 is not expressed in the liver but has a ubiquitous extrahepatic distribution, and is found at high levels in tissues that also accumulate either MEL or 6-HMEL, such as intestine and cerebral cortex, where it may assist in regulating levels of MEL and 6-HMEL.

Our perception of the pineal hormone melatonin (MEL) has changed greatly over the past decade. MEL is the principal substance synthesized and secreted by the pineal gland during the dark period. Its physiological roles in humans are thought to embrace diurnal rhythm, sleep, mood, immunity, reproduction, intestinal motility, and metabolism (Arendt et al., 1999; Skwarlo-Sonta, 2002; Delargrange et al., 2003), and these effects are considered to be mediated by three specific MEL receptor subtypes, namely MT1, MT2, and the putative MT3 binding site (Barrenetxe et al., 2004; Serle et al., 2004). More importantly, there can be little doubt that millions of Americans routinely use dietary supplements, such as MEL, to a wide variety of conditions, both serious and benign.

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Both endogenous and administered MEL are generally considered to be metabolized in humans principally by 6-hydroxylation, with O-demethylation representing a relatively minor pathway. The resulting 6-hydroxymelatonin (6-HMEL) and N-acetyl-5-hydroxytryptamine (N-acetylserotonin, NAS) are excreted in urine as their sulfate and glucuronide conjugates (Young et al., 1985) (see Fig. 1). It has been reported that CYP1A1 (Yeleswaram et al., 1999), CYP1A2 (Yeleswaram et al., 1999; von Bahr et al., 2000; Hartter et al., 2001a,b; Skene et al., 2001; Ursing et al., 2003), and CYP2C19 (von Bahr et al., 2000; Hartter et al., 2001b) all contribute to the metabolism of MEL, but in some cases, the evidence is less than compelling. Detailed knowledge of the hepatic metabolism of MEL is important regarding its administration. However, MEL is present in many tis-

ABBREVIATIONS: MEL, melatonin; 6-HMEL, 6-hydroxymelatonin; 6-CMEL, 6-chloromelatonin; NAS, N-acetylserotonin (N-acetyl-5-hydroxytryptamine); TMS, (E)-2,4,3',5'-tetramethoxy stilbene; WT, wild-type; P450, cytochrome P450; PBS, phosphate-buffered saline; LC-MS/MS, liquid chromatography-tandem mass spectrometry.
sues that are not endowed with the full complement of hepatic cytochromes P450 (P450s); thus, tissue levels may not necessarily be a function of rates of hepatic metabolism or even directly related to plasma concentrations (Reiter and Tan, 2003). MEL is synthesized in the pineal gland, the retina, and the ciliary body (Martin et al., 1992) during the dark cycle, whereby it conveys its chronobiologic properties. Evidence is accumulating that many other tissues, for example, gastrointestinal and skin cells, may have the ability to synthesize MEL (Reiter and Tan, 2003). If the purpose of this synthesis is to reap some local benefit from the action of MEL, it would seem reasonable to propose that these synthetic tissues would also have the ability to inactivate MEL through the process of metabolism. Unfortunately, the tissues that appear to elaborate high concentrations of MEL do not possess significant CYP1A1 or CYP1A2 constitutive activity. We have therefore undertaken a reinvestigation of the metabolism of MEL by individual cDNA-expressed P450s and demonstrate that the ubiquitous extrahepatic P450 CYP1B1 is a principal melatonin 6-hydroxylase.

Materials and Methods

Chemicals and Enzymes. MEL, 6-HMEL, 6-chloromelatonin (6-CMEL), NAS, and NADPH were purchased from Sigma-Aldrich (St. Louis, MO). (E)-2,4,3'-tetramethoxy-stilbene (TMS) was purchased from Calbiochem (San Diego, CA). High-performance liquid chromatography solvents and other chemicals were of the highest grade commercially available. Recombinant human P450 isozymes with P450 reductase, and P450 insect control microsomes were bought from BD Gentest (Woburn, MA).

Incubation Reactions with Recombinant P450 Isoenzymes. Cytochrome P450 panel incubations were carried out in 20 mM phosphate-buffered saline (PBS), pH 7.4, containing 2 pmol of each cDNA-expressed P450 enzyme (insect control, CYP1A1, CYP1A2, CYP1B1, CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4, and CYP4A11) and 10 μM MEL in a final volume of 200 μl. After 5 min of preincubation at 37°C, the reaction was initiated by the addition of 20 μl of 10 mM NADPH and continued for 10 min with shaking. The same experiment was performed without NADPH. Incubation was terminated by the addition of 1 ml of ethyl acetate, followed by 1 ml of 10 mM NADPH and continued for 10 min with shaking. The same experiment was performed without NADPH. Incubation was terminated by the addition of 1 ml of ethyl acetate, followed by 1 ml of 10 mM NADPH and continued for 10 min with shaking. The same experiment was performed without NADPH. Incubation was terminated by the addition of 1 ml of ethyl acetate, followed by 1 ml of 10 mM NADPH and continued for 10 min with shaking. The same experiment was performed without NADPH.

LC/MS/MS Determination of 6-HMEL and NAS. The ethyl acetate-treated incubation mixture was vortexed for 1 min and then centrifuged at 3500g for 5 min. The supernatant was transferred to a new vial and blown to dryness under N2. The extracts were reconstituted with 100 μl of 70% aqueous acetonitrile, and 10 μl was injected for LC/MS/MS analysis. LC/MS/MS analysis was performed on an Applied Biosystems API 2000 ESI triple-quadrupole mass spectrometer (Applied Biosystems, Foster City, CA), using Analyst software, by a modification of our previous method (Yu et al., 2003). A Luna 3-μm C18 50 mm × 46 mm internal diameter column (Phenomenex, Torrance, CA) was used to separate MEL, 6-HMEL, and 6-CMEL (internal standard). A Luna 3-μm C18 50 mm × 46 mm internal diameter column (Phenomenex, Torrance, CA) was used to separate MEL, 6-HMEL, and 6-CMEL (internal standard). A Luna 3-μm C18 50 mm × 46 mm internal diameter column (Phenomenex, Torrance, CA) was used to separate MEL, 6-HMEL, and 6-CMEL (internal standard). A Luna 3-μm C18 50 mm × 46 mm internal diameter column (Phenomenex, Torrance, CA) was used to separate MEL, 6-HMEL, and 6-CMEL (internal standard). A Luna 3-μm C18 50 mm × 46 mm internal diameter column (Phenomenex, Torrance, CA) was used to separate MEL, 6-HMEL, and 6-CMEL (internal standard). A Luna 3-μm C18 50 mm × 46 mm internal diameter column (Phenomenex, Torrance, CA) was used to separate MEL, 6-HMEL, and 6-CMEL (internal standard).

Data Analysis. Results are expressed as the mean ± S.D. Brain homogenates were analyzed in duplicate and recombinant P450s in triplicate. Means were compared using unpaired Student’s t tests with GraphPad QuickCalc on-line (http://www.graphpad.com/quickcalc/index.cfm). Michaelis-Menten parameters were estimated by nonlinear hyperbolic regression (Prism 3.02; GraphPad Software Inc., San Diego, CA).

Results

A novel LC/MS/MS assay was developed for the determination of MEL 6-hydroxylation that uses 6-CMEL as internal standard. The assay is complete within 3 min with the separation of 6-HMEL and 6-CMEL (Fig. 2). The plot of peak area ratio (6-HMEL/6-CMEL peak areas) versus 6-HMEL concentration was linear (r² = 0.9996) up to
5 μM (Fig. 2), with a limit of sensitivity of 0.2 pmol on-column (10 μl of a 20 nM solution), and within-assay and day-to-day variances (at 100 nM 6-HMEL) both < 5%. Incubation of MEL with 11 different human cDNA-expressed P450s revealed that 6-hydroxylation of MEL is mediated by CYP1A1, CYP1A2, CYP1B1, and, to a minor extent, by CYP2C19 (Fig. 3A). All other P450s tested had little or no activity for MEL 6-hydroxylation. The activities for the three CYP1 isozymes were NADPH-dependent. These experiments also revealed that MEL O-demethylation to NAS was, in stark contrast to 6-hydroxylation, mediated by CYP2C19, with a minor contribution from CYP1A2 (Fig. 3B). Michaelis-Menten kinetics for MEL 6-hydroxylation by CYP1A1, CYP1A2, and CYP1B1, conducted up to 200 μM (Fig. 4), gave kinetic constants as shown in Table 1. The $K_{m}$ values were similar for each isozyme, in the range 19 to 31 μM, and not statistically different from one another. However, the turnovers of 6-HMEL by each CYP1, as measured by the $k_{cat}$ values, were in the order CYP1A2 > CYP1A1 = CYP1B1, with CYP1A2 having a turnover that is 1.2 to 2.4 times that of CYP1A1 and CYP1B1, respectively. Kinetics for CYP2C19 were not determined. These findings suggest that, in liver microsomes and in vivo, CYP1A2 is probably the major P450 that mediates the principal metabolic pathway for MEL, 6-hydroxylation. Several anti-CYP1B1 antibodies were available to us, but none possessed good inhibitory characteristics. Therefore, the inhibition of recombinant CYP1B1-mediated MEL 6-hydroxylation was evaluated using the chemical inhibitor TMS, which had been reported to be a selective and potent CYP1B1 inhibitor (Chun et al., 2001; Guengerich et al., 2003). The MEL 6-hydroxylation activity versus log[TMS concentration] curve is shown in Fig. 5. The IC$_{50}$ of TMS for MEL 6-hydroxylation by recombinant human CYP1B1 expressed in baculovirus was estimated to be 30 nM. This compares with IC$_{50}$ values of 6 nM and 90 nM for ethoxyresorufin O-deethylation and estradiol 4-hydroxylation, respectively, by human recombinant CYP1B1 expressed in a bacterial biocstic membrane expression system, and 390 nM for reconstituted purified human CYP1B1 (Chun et al., 2001). CYP1B1-mediated MEL 6-hydroxylation was then investigated in an organ that expresses CYP1B1, together with other CYP1 forms. Mouse brain was used because this organ was reported to express all three CYP1 forms (Granberg et al., 2003; Iba et al., 2003). In addition, whole brain homogenates were used for these studies in light of a report that CYP1B1 may be expressed in the nuclei of brain and other tissues (Muskhelishvili et al., 2001). As shown in Fig. 6, brain homogenate from cyp1b1-null mice had approximately 40% lower activity ($t = 13.0; P < 0.006$) of MEL 6-hydroxylation (110 ± 5.4 pmol min$^{-1}$ g$^{-1}$ wet tissue) than brain homogenate from WT mice (182 ± 5.6 pmol min$^{-1}$ g$^{-1}$ wet tissue). Moreover, the addition of 100 nM TMS to WT brain homogenate inhibited MEL 6-hydroxylation by 27% to 132% (WT: $t = 10.1; P = 0.01$), and this value was only marginally statistically significantly different ($t = 4.59; P = 0.044$) from the activity in the cyp1b1-null brain homogenate. Thus, ~40% of the MEL 6-hydroxylation activity of mouse brain homogenate can be attributed to CYP1B1 and is sensitive to inhibition by TMS.

**Discussion**

This first study of MEL 6-hydroxylation using recombinant human P450s confirms earlier reports (Yeleswaram et al., 1999; von Bahr et al., 2000; Hartter et al., 2001a,b; Skene et al., 2001; Ursing et al., 2003) that CYP1A2 is an important isozyme for MEL 6-hydroxylation. The finding that CYP1A1 also 6-hydroxylates MEL confirms a single report (Yeleswaram et al., 1999), which also used recombinantly expressed human P450s, but which only determined the rate of disappearance of substrate. However, this is also the first report that CYP1B1 6-hydroxylates MEL. It is of interest to inquire why this has hitherto not been reported, despite the several studies of MEL metabolism. First, in the human in vivo studies, it was inferred that CYP1A2 was the principal enzyme metabolizing MEL by the use of CYP1A2 inhibitors caffeine and fluvoxamine (von Bahr et al., 2000; Hartter et al., 2001a; Skene et al., 2001; Ursing et al., 2003). Whereas caffeine 3-demethylation is principally carried out by CYP1A2, CYP1B1 possesses about 30% of this activity and 8-hydroxylates caffeine almost as well as CYP1A2 (Shimada et al., 1997). Thus, caffeine administration might be expected to inhibit CYP1B1 in addition to CYP1A2. We could find no published reports on the effect of fluvoxamine on CYP1B1 activity. Constitutive expression of CYP1A2 would also be expected to exceed
that of CYP1B1, particularly in liver. Therefore, the human in vivo studies do not exclude the role of CYP1B1 in the metabolism of MEL. Second, regarding the in vitro studies, the first study using recombinant P450s with MEL (Yeleswaram et al., 1999) reported that MEL inhibited recombinant CYP1A2-mediated 3-cyano-7-ethoxycoumarin O-deethylase activity with a low IC_{50} (ca. 20 μM). Incubation of MEL with various recombinant human P450s for 3 h was reported to yield a <5% disappearance of MEL from the incubation, except for CYP1A1 and CYP1A2, where 90 and 92%, respectively, had been metabolized. Although recombinant CYP1B1 was available at this time (Shimada et al., 1997), it was not investigated by these authors (Yeleswaram et al., 1999). Human liver microsomes have been reported to 6-hydroxylate MEL and the activity is completely inhibited by furafylline, strongly suggesting that CYP1A2 mediated this activity (Hartter et al., 2001b). These same authors reported that recombinant CYP2C19 did not 6-hydroxylate MEL, contrary to the present finding of a minor role for CYP2C19 in this regard, despite using  

![Table 1](image)

**TABLE 1**  
Michaelis-Menten parameters estimated for melatonin 6-hydroxylation by individual cDNA-expressed CYP1 family isozymes  

<table>
<thead>
<tr>
<th>CYP1 Isozyme</th>
<th>( V_{\text{max}} ) (pmol min^{-1})</th>
<th>( K_m ) (μM)</th>
<th>( V_{\text{max}}/K_m ) (μmol min^{-1} P450)</th>
<th>( R^2 )</th>
<th>( IC_{50} ) (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A1</td>
<td>6.46 ± 0.22</td>
<td>19.2 ± 2.01</td>
<td>0.33</td>
<td>0.982</td>
<td></td>
</tr>
<tr>
<td>CYP1A2</td>
<td>10.6 ± 0.32</td>
<td>25.9 ± 2.47</td>
<td>0.41</td>
<td>0.989</td>
<td></td>
</tr>
<tr>
<td>CYP1B1</td>
<td>5.31 ± 0.21</td>
<td>30.9 ± 3.76</td>
<td>0.17</td>
<td>0.984</td>
<td></td>
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**FIG. 3.** Relative rates of melatonin 6-hydroxylation (A) and O-demethylation (B) by 11 cDNA-expressed human P450s and control insect microsomes. Supersomes containing each P450 were incubated with melatonin, and the production of the 6-hydroxy and O-demethylated metabolites was determined by LC-MS/MS.  

**FIG. 4.** Velocity curves for melatonin 6-hydroxylation for CYP1 forms. Kinetics of melatonin 6-hydroxylation was determined for recombinant CYP1A1, CYP1A2, and CYP1B1 using concentrations of melatonin ranging from 0.1 to 200 μM.  

**FIG. 5.** Determination of the IC_{50} value for the selective CYP1B1 inhibitor TMS versus melatonin 6-hydroxylation by recombinant CYP1B1. CYP1B1 Supersomes were incubated with logarithmic incremental concentrations (0.1–1000 nM) of the inhibitor TMS, and the IC_{50} value of TMS for melatonin 6-hydroxylation by CYP1B1 was determined.
recombinant CYP2C19 from the same source as us. It should be noted that this study did not consider a role for CYP1B1 in melatonin metabolism. However, it was postulated (von Bahr et al., 2000) that CYP2C19 may 6-hydroxylate MEL based upon the report that flavoxamine, an inhibitor of CYP1A2 but also, to some extent, CYP2C19, elevated almost 3-fold the area under the plasma concentration-time curve for endogenous MEL. Such pharmacokinetic findings may act as a signpost but cannot be taken as definitive evidence for the role of CYP2C19 in MEL 6-hydroxylation. To clarify further the role of CYP2C19 in MEL metabolism, a panel of 11 recombinant P450s was used to investigate the minor metabolic pathway of MEL, O-demethylation to produce N-acetylsertotonin (reaction B in Fig. 1). These results revealed that this reaction is mediated principally by CYP2C19, with a small contribution from CYP1A2, in contrast to the 6-hydroxylation pathway. Thus, the hepatic metabolism of melatonin in vivo likely involves mainly CYP1A2 with some contribution from CYP2C19. These findings are in harmony with the in vivo studies (von Bahr et al., 2000; Hartter et al., 2001a; Ursing et al., 2003).

Finally, rat liver microsomes and liver slices were previously used to study the conversion of MEL to the sulfate conjugate of 6-HMEL (Skinner and Malpaux, 1999). Rats treated with β-naphthoflavone had a 3-fold increase in activity and, again, it was assumed that this was due entirely to the induction of CYP1A2 in the liver. However, β-naphthoflavone also induces CYP1B1 in hepatocyte cell lines (Krusekopf et al., 2003). Again, a role for CYP1B1 cannot be excluded in this report.

All the accumulated data suggest that CYP1A2 plays a major role in MEL hepatic clearance, with a minor contribution from CYP2C19. However, our knowledge of the biology of MEL is rapidly growing, and it would seem that MEL reaches concentrations in some tissues that are significantly greater than its plasma concentrations. In regions of the brain (Skinner and Malpaux, 1999), in skin and intestine (Reiter and Tan, 2003), MEL reaches levels up to 20-fold higher than nocturnal plasma concentrations. Moreover, in mice fed a diet containing 40 ppm MEL for 11 weeks, 99.9% of MEL in the cerebral cortex had been 6-hydroxylated and then conjugated with sulfate, compared with 75% in the plasma compartment (Lahiri et al., 2004). In untreated mice, free MEL concentrations in serum were 1 to 2 orders of magnitude higher than in liver, kidney, heart, and cerebral cortex. However, heart and cerebral cortex displayed 30- to 50- and 60- to 1400-fold excesses of 6-HMEL, respectively. Uninduced rat cerebral cortex contains relatively little CYP1A2, but this can be induced considerably by β-naphthoflavone treatment (Iba et al., 2003). CYP1A2 is unlikely to be the source of the massively elevated MEL metabolite levels in the cerebral cortex. On the other hand, various elements of the mouse blood-brain barrier, including the cerebral arteries and arterioles, have been reported to have very high levels of CYP1B1 that apparently were not further inducible (Granberg et al., 2003). It is possible that CYP1B1 played a role in the sequestration of high concentrations of 6-HMEL sulfate in mouse cerebral cortex. Other adult mouse tissues that have been found to display high expression of CYP1B1 include heart, whole brain, spleen, lung, kidney, and testis (Choudhary et al., 2003). It is noteworthy that kidney and heart, in addition to the cerebral cortex, are two organs that accumulate 6-HMEL sulfate (Lahiri et al., 2004). The direct evidence presented in this study establishes a role for CYP1B1 in the 6-hydroxylation of MEL in mouse brain homogenerate. This evidence is 2-fold: first, whole brain homogenerate from male WT mice had a statistically significantly higher rate (P < 0.006) of MEL 6-hydroxylation (182 ± 5.6 pmol min⁻¹ g⁻¹ wet tissue) than whole brain homogenerate from male cyp1b1-null mice (110 ± 5.4 pmol min⁻¹ g⁻¹ wet tissue), suggesting that CYP1B1 is responsible for approximately 40% of MEL 6-hydroxylation in mouse brain. Second, a 100 nM concentration of the CYP1B1-selective inhibitor TMS decreased MEL 6-hydroxylase activity of WT mouse brain homogenerate by 27%. Although this inhibitor concentration is only 3.3 times the IC₅₀ value of 30 nM and, from Fig. 5, might be expected to produce inhibition of the order of 80%, we did not use higher concentrations because it has been reported that TMS also inhibits CYP1A1 and CYP1A2 at higher concentrations. The reported IC₅₀ values for ethoxyresorufin O-deethylase inhibition by TMS for human recombinant CYP1A1, CYP1A2, and CYP1B1 were 300, 3000, and 6 nM, respectively (Chun et al., 2001). It should be noted that mouse CYP1B1 and not human CYP1B1 was used with melatonin, and not ethoxyresorufin as a substrate. The data presented in Fig. 6 show that the inhibited activity in WT brain (132 ± 4.1 pmol min⁻¹ g⁻¹ tissue) approaches that of the cyp1b1-null brain (110 ± 5.4 pmol min⁻¹ g⁻¹ tissue), but not completely, at the inhibitor concentration of 100 nM. The difference was only marginally statistically significantly different (P = 0.044). This is a second line of evidence that MEL is 6-hydroxylated by CYP1B1 in mouse brain.

Based on the findings described here and on the developing scientific literature, it appears that CYP1A2 and, to some extent, CYP2C19 are largely responsible for the plasma clearance of MEL by the liver. In addition, the present study would indicate that CYP1B1, which is not significantly expressed in liver but which has a more ubiquitous distribution in mouse brain elements of the mouse blood-brain barrier, including the cerebral arteries and arterioles, have been reported to have very high levels of CYP1B1 that apparently were not further inducible (Granberg et al., 2003). It is possible that CYP1B1 played a role in the sequestration of high concentrations of 6-HMEL sulfate in mouse cerebral cortex. Other adult mouse tissues that have been found to display high expression of CYP1B1 include heart, whole brain, spleen, lung, kidney, and testis (Choudhary et al., 2003). It is noteworthy that kidney and heart, in addition to the cerebral cortex, are two organs that accumulate 6-HMEL sulfate (Lahiri et al., 2004). The direct evidence presented in this study establishes a role for CYP1B1 in the 6-hydroxylation of MEL in mouse brain homogenerate. This evidence is 2-fold: first, whole brain homogenerate from male WT mice had a statistically significantly higher rate (P < 0.006) of MEL 6-hydroxylation (182 ± 5.6 pmol min⁻¹ g⁻¹ wet tissue) than whole brain homogenerate from male cyp1b1-null mice (110 ± 5.4 pmol min⁻¹ g⁻¹ wet tissue), suggesting that CYP1B1 is responsible for approximately 40% of MEL 6-hydroxylation in mouse brain. Second, a 100 nM concentration of the CYP1B1-selective inhibitor TMS decreased MEL 6-hydroxylase activity of WT mouse brain homogenerate by 27%. Although this inhibitor concentration is only 3.3 times the IC₅₀ value of 30 nM and, from Fig. 5, might be expected to produce inhibition of the order of 80%, we did not use higher concentrations because it has been reported that TMS also inhibits CYP1A1 and CYP1A2 at higher concentrations. The reported IC₅₀ values for ethoxyresorufin O-deethylase inhibition by TMS for human recombinant CYP1A1, CYP1A2, and CYP1B1 were 300, 3000, and 6 nM, respectively (Chun et al., 2001). It should be noted that mouse CYP1B1 and not human CYP1B1 was used with melatonin, and not ethoxyresorufin as a substrate. The data presented in Fig. 6 show that the inhibited activity in WT brain (132 ± 4.1 pmol min⁻¹ g⁻¹ tissue) approaches that of the cyp1b1-null brain (110 ± 5.4 pmol min⁻¹ g⁻¹ tissue), but not completely, at the inhibitor concentration of 100 nM. The difference was only marginally statistically significantly different (P = 0.044). This is a second line of evidence that MEL is 6-hydroxylated by CYP1B1 in mouse brain.

Based on the findings described here and on the developing scientific literature, it appears that CYP1A2 and, to some extent, CYP2C19 are largely responsible for the plasma clearance of MEL by the liver. In addition, the present study would indicate that CYP1B1, which is not significantly expressed in liver but which has a more ubiquitous extrahepatic distribution, is for the most part accountable for MEL 6-hydroxylation outside the liver. At present, the roles of MEL in many organs are not fully understood. The finding that certain tissues appear to accumulate MEL metabolites in the mouse has yet to be reproduced in humans. The role of CYP1A1 and CYP1B1 in the physiology of MEL remains to be revealed.

References


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