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Contribution of CYP1A2 in the hepatic metabolism of melatonin: studies with isolated microsomal preparations and liver slices

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Abstract: The objective of the present studies was to define the enzyme systems catalysing the 6-hydroxylation of melatonin, by monitoring the levels of 6-sulphatoxymelatonin in rat hepatic postmitochondrial preparations and in precision-cut liver slices. Melatonin 6-hydroxylase activity was localized in microsomes and was supported by NADPH, but not NADH. Treatment of rats with β-naphthoflavone more than tripled 6-sulphatoxymelatonin formation from melatonin, but gave rise only to a moderate increase (25%) in the sulphate conjugation of 6-hydroxymelatonin. Treatment of rats with phenobarbitone, acetone, dexamethasone and clofibrate did not increase 6-sulphatoxymelatonin generation when either melatonin or 6-hydroxymelatonin served as substrates. Of a number of cytochrome P450 inhibitors investigated, only furafylline inhibited markedly the conversion of melatonin to 6-sulphatoxymelatonin without any concomitant effect on the sulphoconjugation of 6-hydroxymelatonin. When liver slices were incubated with melatonin, treatment of rats with β-naphthoflavone, and to a lesser extent phenobarbitone, elevated the levels of 6-sulphatoxymelatonin in the culture medium. No such increase was seen when slices from β-naphthoflavone-treated rats were incubated with 6-hydroxymelatonin, whereas a modest increase was seen with slices from phenobarbitone-treated rats. Treatment of rats with acetone, dexamethasone or clofibrate failed to modulate the levels of 6-sulphatoxymelatonin generated from either melatonin or 6-hydroxymelatonin. Molecular modelling analysis revealed that melatonin had a high area/depth^2 ratio, displayed characteristics of CYP1A2 substrates and could be readily accommodated into the human CYP1A2 active site in a position favouring 6-hydroxylation. Collectively, all the above data provide strong experimental evidence that CYP1A2 is an important catalyst of the 6-hydroxylation of melatonin.

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Introduction

Melatonin (5-methoxy-N-acetyltryptamine) is a hormone secreted by the pineal gland; its synthesis and secretion display a regular circadian rhythm with maximum levels being produced during the dark phase. It plays a major role in a number of important physiological functions such as regulation of circadian rhythms and seasonal reproduction [Arendt, 1995; Borjigin et al., 1999]. This ability of melatonin to phase shift circadian rhythms led to its use in the treatment of conditions resulting from biological rhythms disturbances, such as jet lag following rapid time-zone changes and shift work [Skene et al., 1996; Arendt et al., 1997]. Melatonin is available over the counter and is widely consumed in many countries, particularly in the USA [Bonn, 1996]. It is conceivable that this use of melatonin may lead to adverse drug interactions in patients who also take other medication. Indeed, such interactions have already been reported, in both animals and humans,

involving drugs such as fluvoxamine and 5-methoxypsoralen but the underlying mechanisms have not been clarified [Mauviard et al., 1991; Skene et al., 1994; Härtter et al., 2000]. In order for such drug interactions to be predicted, and thus avoided, it is imperative that the nature of the enzyme(s) responsible for the metabolism of melatonin is fully defined.

Melatonin is rapidly metabolized, the half-life of circulating melatonin in humans, following exogenous administration, ranging between 10 and 60 min [Waldhauser et al., 1984]. The principal enzymic metabolic pathway involves hydroxylation at the 6-position; the hydroxylated metabolite being excreted largely as the sulphate conjugate and, to a lesser extent, as the glucuronide, with very small amounts in the unconjugated form [Arendt et al., 1985]. The enzyme system(s) responsible for the 6-hydroxylation of melatonin have not been systematically investigated. In the present paper we present evidence for the involvement of microsomal cytochromes P450 in the metabolism of pharmacological concentrations of melatonin, and we identify CYP1A2 as the principal catalyst of the 6-hydroxylation of melatonin. Studies were performed using isolated microsomal papers and precision-cut liver slices; in the latter system cytochrome P450 oxidation and phase II conjugation reactions are coupled, and structural architecture is maintained.

Materials and methods

Melatonin, 6-hydroxymelatonin, insulin, hydrocortisone 21-hemisuccinate, dexamethasone, phenobarbitone, clofibrate, erythromycin, diethylthiocarbamate, α- and β-naphthoflavone and all cofactors (Sigma, Poole, Dorset, UK), Earles balanced salt solution (EBSS), foetal calf serum and RPMI 1640 culture media (Gibco, Paisley, Scotland, UK), Corning 12-well plates (Western Laboratory Services, Aldershot, Hampshire, UK), furafylline, (S)-(+)mephenytoin, ketoconazole and quinidine (Ultrafine, Manchester, UK) were all purchased. The antibody to 6-sulphatoxymelatonin, raised in sheep, was a generous gift from Stockgrand Ltd., University of Surrey, Guildford, UK.

Male Wistar albino rats (200–250 g) were purchased from B&K Universal, Ltd. (Hull, East Yorkshire, UK), and housed in a 12:12 hr light:dark cycle (LD; lights on at 06.00 h). Groups of four rats were treated with the following cytochrome P450 inducing agents; single daily intraperitoneal doses of acetone (100 mg/kg), dexamethasone (30 mg/kg), β-naphthoflavone (25 mg/kg), phenobarbitone (80 mg/kg) or clofibrate (80 mg/kg) for 3 days, all animals being killed 24 hr after administration. Livers were immediately excised, homogenized in 1.15% KCl (25% w/v homogenate), and subcellular fractions were prepared by differential centrifugation as previously described [Ioannides and Parke, 1975], or precision-cut slices were prepared [Hashemi et al., 1999].

Studies with subcellular fractions

Determination of melatonin 6-hydroxylase activity in hepatic preparations was achieved by measuring its sulphate conjugate using the following incubation system in a total volume of 0.5 mL: potassium phosphate buffer, pH 7.4 (25 μmol), glucose 6-phosphate (7.5 μmol), NADP (1 μmol), adenosine 3′-phosphate 5′-phosphosulphate (PAPS, 50 nmol) and 50 μL of hepatic postmitochondrial fraction (or any other hepatic fraction). After an initial preincubation of 10 min at 37°C, reaction was initiated by the addition of melatonin (25 nmol) and the mixture was incubated in a shaking waterbath at 37°C for 20 min, unless otherwise stated. Reaction was terminated by the addition of 0.2 M perchloric acid (250 μL) and protein was precipitated by centrifugation at 2500g for 15 min. The levels of 6-sulphatoxymelatonin were determined using a previously described radioimmunoassay procedure [Aldhous and Arendt, 1988]. The sulphate conjugation of 6-hydroxymelatonin was determined using the above incubation procedure, except that melatonin was replaced with its 6-hydroxy metabolite. Protein in the various hepatic fractions was assayed using bovine serum albumin as standard [Lowry et al., 1951].

In the studies employing cytochrome P450 inhibitors, the inhibitor was added to the incubation system with the corresponding volume of the solvent vehicle being added to control tubes. In the case of furafylline, diethylthiocarbamate and erythromycin, a 20-min preincubation was carried out to allow the inhibitors to be converted to their cytochrome P450-binding metabolites.

Studies with liver slices

Hepatic slices were prepared using a Krumdieck tissue slicer (Alabama Research and Development Corporation, Munsford, AL) as previously described [Hashemi et al., 1999]. The multiwell plate procedure, employing 12-well culture plates, was used to culture the slices. The culture medium was essentially that described by Lake et al., [1993] and comprized RPMI 1640 containing foetal calf

serum (5%), L-methionine (0.5 mM), insulin (1 μM), gentamicin (50 μg/mL) and hydrocortisone 21-hemisuccinate (0.1 mM). One slice was placed in each well, in 1.5 mL of culture medium. Slices were incubated under sterile conditions on a gyratory shaker housed in a humidified incubator, at a temperature of 37°C and under an atmosphere of 5% CO_2/95% air. Following an initial 15-min preincubation, the medium was replaced with fresh medium containing melatonin (50 μM) and incubation was carried out for various time periods. The slice was then removed from the incubation and the medium was stored at −20°C. 6-Sulphatoxymelatonin was determined in the culture medium using a radioimmunoassay procedure [Aldhous and Arendt, 1988].

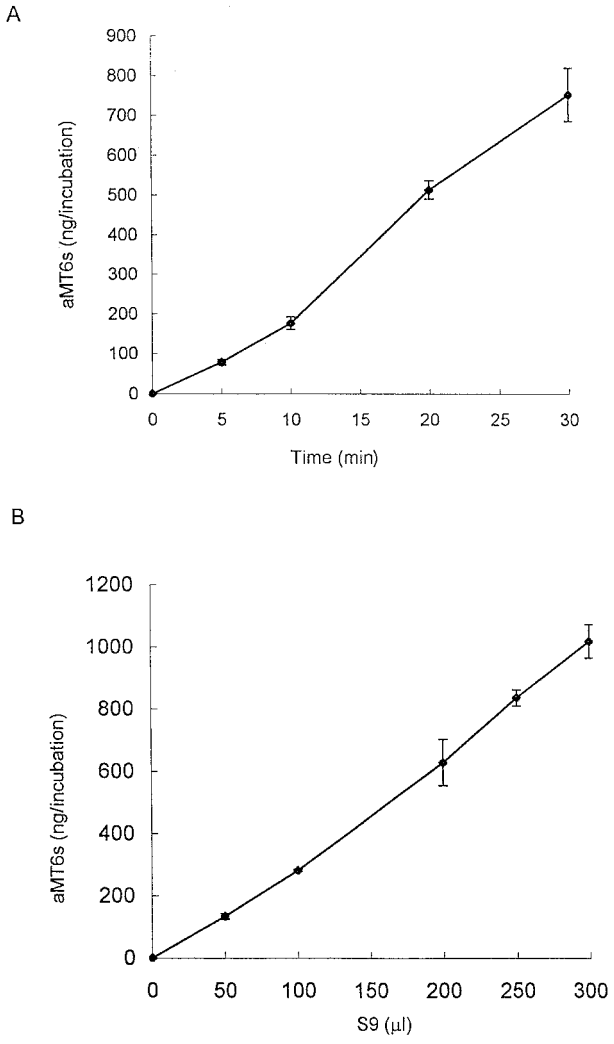

Fig. 1. Time- and postmitochondrial supernatant concentration-dependent metabolism of melatonin to 6-sulphatoxymelatonin. Melatonin (50 μM) was incubated for different time periods (**A**) and with a range of hepatic postmitochondrial concentrations (S9) (**B**) isolated from untreated rats. Results are presented as mean ± S.E.M. for triplicates.

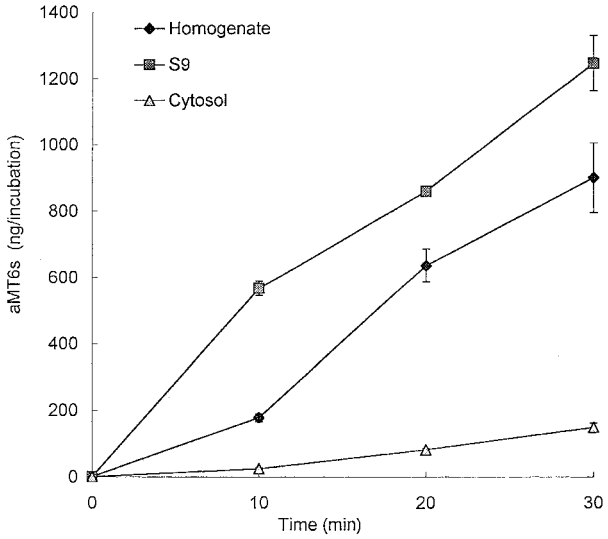

Fig. 2. Metabolism of melatonin to 6-hydroxymelatonin by hepatic homogenate and subfractions. Melatonin (50 μM) was incubated with total liver homogenate, postmitochondrial (S9) and cytosolic fractions (25% w/v) isolated from untreated animals. Results are presented as mean ± S.E.M. for triplicates.

Molecular modelling

The melatonin structure was built using the Sybyl fragment library, and modelled on the basis of the indole fragment. The energy-minimized substrate was then docked interactively within the putative site of human CYP1A2 [Lewis et al., 1999] using the Sybyl molecular modelling software (Tripos Associates, St Louis, MO), which was also used for calculating the molecular dimensions of melatonin. The interactive docking procedure was facilitated by knowledge of the preferred orientations of other CYP1A2 substrates [Lewis et al., 1999] and by the known site of metabolism of melatonin, namely 6-hydroxylation.

Statistical evaluation was performed using one way ANOVA followed by the Tukey post-hoc test.

Results

The generation of 6-sulphatoxymelatonin was linear with time for at least 30 min and was dependent on the concentration of the postmitochondrial preparation in the incubation (Fig. 1). Melatonin was readily converted to the 6-sulphatoxymetabolite by liver homogenate and postmitochondrial preparations, but no significant activity was observed in the cytosolic fraction (Fig. 2). The postmitochondrial metabolism of melatonin was supported by NADPH but not by NADH (Table 1); replacement of the oxygen with nitrogen caused a marked decrease in

Table 1. Cofactor and oxygen requirement for the hepatic metabolism of melatonin

Cofactor (mM)	Gaseous atmosphere	6-Sulphatoxymelatonin (ng/min per mg protein)
Experiment 1		
None	Oxygen	0.0 ± 0.0
NADPH (2)	Oxygen	8.1 ± 0.3
NADH (2)	Oxygen	1.0 ± 0.02
NADPH (2) + NADH (2)	Oxygen	8.1 ± 0.4
Experiment 2		
NADPH (2)	Oxygen	5.6 ± 0.4
NADPH (2)	Nitrogen	2.4 ± 0.5

In addition to the above cofactors, the incubation mixture was supplemented with PAPS as indicated in the text. In the second experiment, nitrogen was bubbled through the incubation mixture for 10 min prior to the addition of melatonin and during the incubation. Results are presented as the mean ± S.E.M. of triplicate determinations.

6-sulphatoxymelatonin production (Table 1). Pretreatment of rats with β-naphthoflavone nearly trebled the formation of 6-sulphatoxymelatonin, whereas treatments with acetone, dexamethasone or phenobarbitone failed to modulate the activity (Fig. 3). Finally, treatment with clofibrate caused a statistically significant inhibition. When 6-hydroxymelatonin served as the substrate, treatment with β-naphthoflavone caused a 25% increase in sulphate conjugation (Table 2).

Of all cytochrome P450 inhibitors studied, only furafylline and α-naphthoflavone caused a marked and concentration-dependent inhibition of metabolism of melatonin to 6-sulphatoxymelatonin (Fig. 4). At the highest concentration only (100 μM), chlorzoxazone also caused a marked inhibition of the formation of 6-sulphatoxymelatonin from melatonin. α-Naphthoflavone, but not furafylline or chlorzoxazone, also inhibited the sulphate conjugation of 6-hydroxymelatonin (Fig. 5). The same picture emerged when the postmitochondrial preparations were derived from β-naphthoflavone-treated rats, in that furafylline and α-naphthoflavone suppressed the production of 6-sulphatoxymelatonin from melatonin, whereas only the flavonoid also inhibited its formation from 6-hydroxymelatonin (Fig. 6).

Table 2. The effect of phenobarbitone and β-naphthoflavone on the sulphate conjugation of 6-hydroxymelatonin

Animal treatment	6-Sulphatoxymelatonin (ng/min per mg protein)
Control	56.5 ± 4.4
Phenobarbitone	51.0 ± 5.0
β-Naphthoflavone	72.3 ± 1.8*

Results are presented as mean ± S.E.M. for four animals. Each analysis was carried out in triplicate.
* $P < 0.05$.

Rat liver slices metabolized melatonin to the 6-sulphatoxymelatonin in a time-dependent fashion (Fig. 7). The generation of 6-sulphatoxymelatonin was enhanced following pretreatment of the animals with β-naphthoflavone, and to a lesser extent by phenobarbitone, whereas no increase was seen with the other cytochrome P450 inducers (Fig. 8). When 6-hydroxymelatonin was incubated with liver slices, sulphate conjugation was elevated following pretreatment with phenobarbitone but not β-naphthoflavone (Fig. 9).

The molecular dimensions of melatonin were calculated and found to be: length 12.8074 Å, width 10.20052 Å and depth 5.73678 Å, giving an area/depth^2 ratio of 3.97. Fig. 10 illustrates the

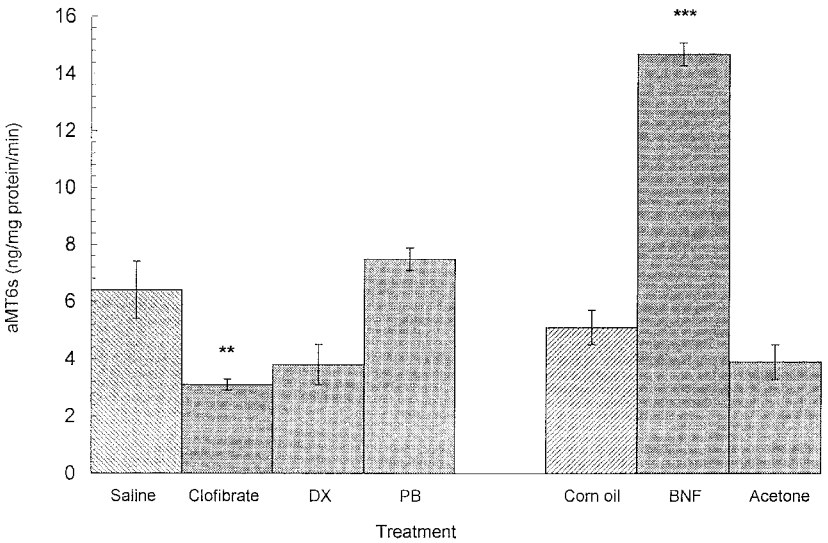

Fig. 3. Effect of cytochrome P450 inducers on the metabolism of melatonin to 6-sulphatoxymelatonin by rat liver postmitochondrial supernatant. Postmitochondrial preparations (25% w/v) from rats pretreated with various cytochrome P450 inducers were incubated with melatonin (50 μM). Results are presented as mean ± S.E.M. for four animals. Each determination is carried out in triplicate. * $P < 0.05$; ** $P < 0.005$; *** $P < 0.001$ when compared to the control group. DX, dexamethasone; PB, phenobarbitone; BNF, β-naphthoflavone.

docking of melatonin within the putative active site of CYP1A2, where a combination of two hydrogen bonds orientates the molecule for hydroxylation at the 6-position, the distance from the haem being 4.153 Å. Two active site threonine residues, Thr78 and Thr87, enter into hydrogen bonding with the amide and methoxy groups of melatonin, respectively. The hydrogen at the 6-position of melatonin is located directly above the haem iron, and the position of the conserved distal threonine (Thr268) is also shown, together with other active site residues known to bind other CYP1A2 substrates. These include the two π-stacking aromatic residues, Phe181 and Tyr437, with an additional threonine (Thr438), which forms bonds with several other CYP1A2 substrates. Phe181 has been shown by site-directed mutagenesis to affect substrate binding [Parikh et al., 1999] and consequently is likely to reside in the active site.

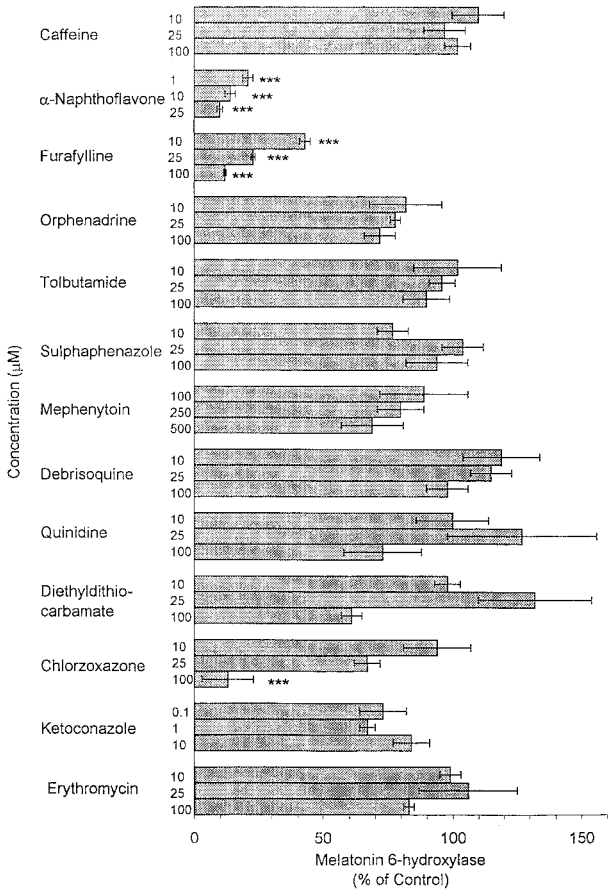

Fig. 4. Effect of cytochrome P450 inhibitors on the transformation of melatonin to 6-sulphatoxymelatonin by rat liver postmitochondrial supernatant. The metabolism of melatonin to 6-sulphatoxymelatonin by hepatic postmitochondrial supernatant was investigated in the presence and absence of cytochrome P450 inhibitors. Results are presented as mean ± S.E.M. for triplicate determinations. Inhibitors that were not water soluble were dissolved in 10 μL of either DMSO or ethanol. The 100% melatonin 6-hydroxylase activity was 10.1 ± 0.6 (n = 15) ng/min per mg protein but decreased to 7.4 ± 0.4 (n = 15) and 7.8 ± 0.6 (n = 9) in the presence of 10 μL of DMSO and ethanol, respectively. * $P < 0.05$; ** $P < 0.005$; *** $P < 0.001$ when compared to the appropriate control.

Discussion

The enzymic formation of 6-hydroxymelatonin, determined as the sulphate conjugate, was linear with time indicating that sulphotransferase activity was not limiting. Melatonin 6-hydroxylase activity was localized in the microsomes, required molecular oxygen and was supported by NADPH, but not NADH, hallmarks of the cytochrome P450-dependent mixed function oxidases. Cytochromes P450 exist as a superfamily of enzymes, each of which is characterized by its own substrate specificity [Ioannides, 1996]. Most xenobiotic-metabolizing cytochrome P450 proteins can be selectively induced by exposure to chemicals. When postmitochondrial liver preparations were used, only the treatment with β-naphthoflavone enhanced significantly the generation of 6-sulphatoxymelatonin, resulting in a 200% rise in activity. In order to ensure that this increase was the consequence of an increase in the 6-hydroxylation of melatonin, rather than in the subsequent sulphate conjugation, studies were undertaken to evaluate whether the same treatment influenced the sulphation of 6-hydroxymelatonin. A modest increase of less than 30% was observed, indicating that the increase in the production of 6-sulphatoxymelatonin following treatment with β-naphthoflavone is largely due to increased cytochrome P450-mediated oxidation. This finding is strongly supported by the studies employing liver slices, in that only the treatment with β-naphthoflavone elevated the levels of 6-sulphatoxymelatonin in the culture media with no effect on sulphation, as no similar increase was evident when 6-hydroxymelatonin served as the substrate. As β-naphthoflavone is a selective inducer of the CYP1A subfamily [Ioannides and Parke, 1990], these observations implicate this subfamily in the 6-hydroxylation of melatonin. It has previously been reported that treatment of rats with the polycyclic aromatic hydrocarbons benzo[a]pyrene or 7,12-dimethylbenz[a]anthracene, selective inducers of the CYP1A subfamily [Ioannides and Parke, 1990], stimulated the 6-hydroxylation of melatonin [Beedham et al., 1987; Praast et al., 1995]. The CYP1A subfamily comprises two proteins, namely CYP1A1 and CYP1A2, but whether one or both of these proteins participate in the 6-hydroxylation

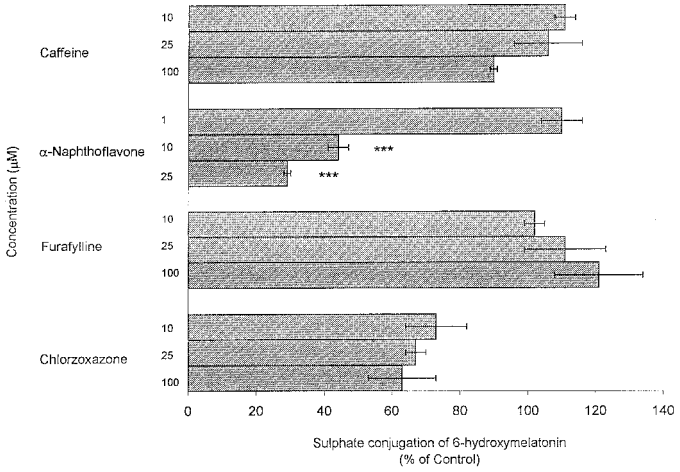

Fig. 5. Effect of cytochrome P450 inhibitors on the sulphate conjugation of 6-hydroxymelatonin by rat liver cytosol. Hepatic cytosol (25% w/v) from untreated rats was incubated with 6-hydroxymelatonin in the presence and absence of cytochrome P450 inhibitors. Results are presented as mean ± S.E.M. for triplicate determinations. Inhibitors that were not water soluble were dissolved in DMSO. The 100% activity was 202 ± 13 (n = 6) ng/min per mg protein but decreased to 160 ± 28 (n = 9) in the presence of 10 μL DMSO. * $P < 0.05$; ** $P < 0.005$; *** $P < 0.001$ when compared to the appropriate control.

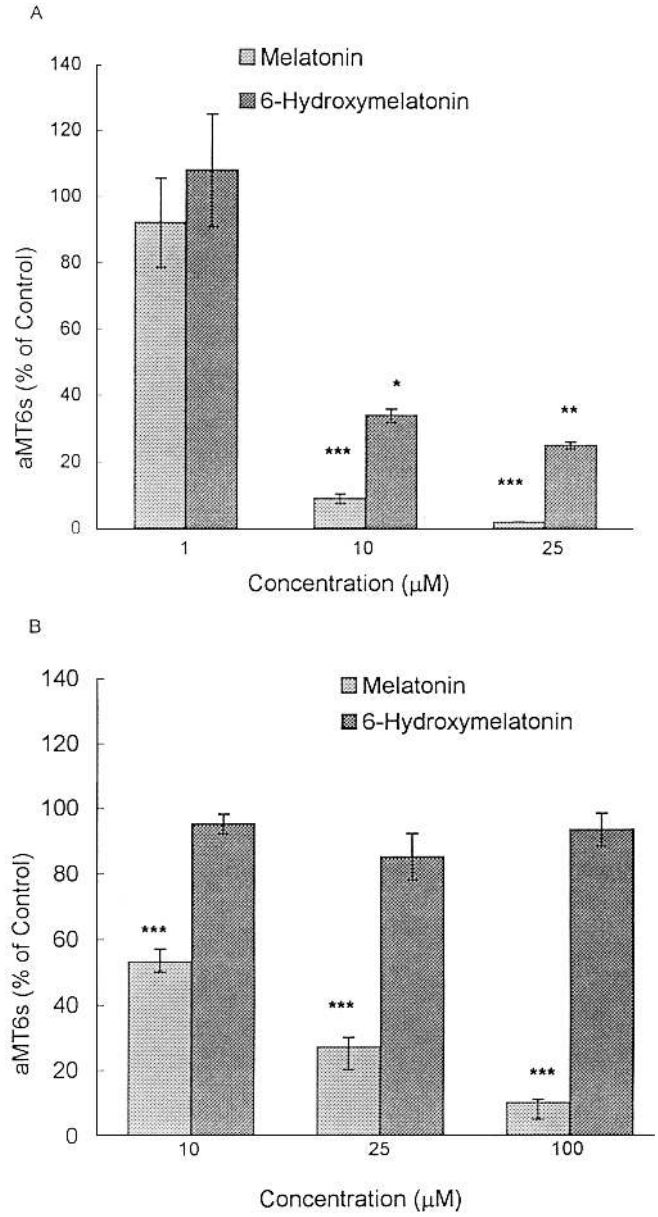

Fig. 6. Effect of furafylline and α-naphthoflavone on the generation of 6-sulphatoxymelatonin from melatonin or 6-hydroxymelatonin by rat liver postmitochondrial supernatant and cytosol, respectively. Hepatic postmitochondrial supernatant or cytosol (25% w/v) from β-naphthoflavone-treated rats was incubated with melatonin and 6-hydroxymelatonin, respectively. The study was carried out in the absence and presence of either α-naphthoflavone (**A**) or furafylline (**B**), both being dissolved in DMSO. Results are presented as mean ± S.E.M. for triplicate determinations. The 100% activities, in the presence of DMSO, for the formation of 6-sulphatoxymelatonin from melatonin and 6-hydroxymelatonin were 37.7 ± 2.2 (n = 6) and 210 ± 20 (n = 6), respectively. * $P < 0.05$; ** $P < 0.005$; *** $P < 0.0001$ compared to the appropriate control.

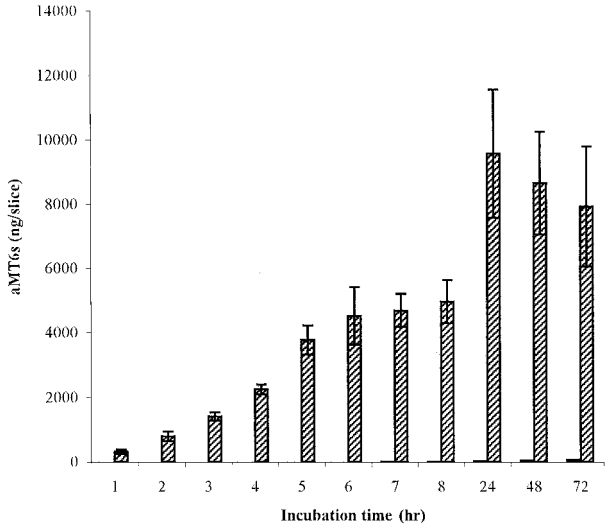

Fig. 7. Cumulative metabolism of melatonin to 6-sulphatoxymelatonin by precision-cut liver slices. Liver slices from untreated rats were cultured in the presence of melatonin (50 μM) for various time periods up to 72 hr. Results are presented as mean ± S.E.M. for three animals. Each culture time was carried out in duplicate and similarly each analysis was performed in duplicate.

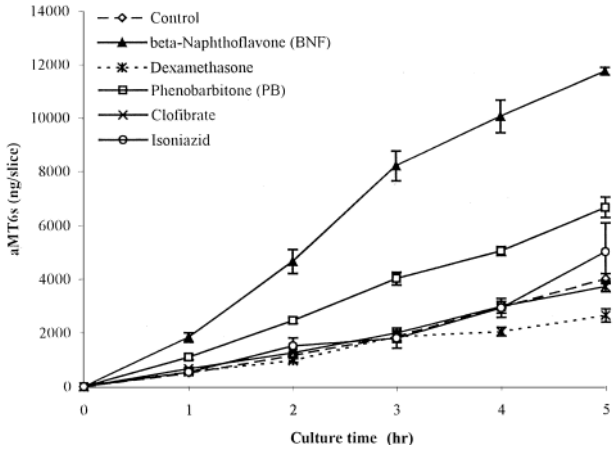

Fig. 8. Effect of cytochrome P450 inducers on the generation of 6-sulphatoxymelatonin from melatonin by precision-cut liver slices. Precision-cut liver slices from animals pretreated with various cytochrome P450 inducers were incubated for various time periods, up to 5 hr, with melatonin (50 μM), and 6-sulphatoxymelatonin was determined in the medium. Results are presented as mean ± S.E.M. for three animals. Each culture time was carried out in duplicate and similarly each analysis was performed in duplicate.

of melatonin cannot be discerned from the above studies.

A statistically significant inhibition in the hepatic postmitochondrial formation of 6-sulphatoxymelatonin was evident in the animals treated with clofibrate. This is a selective inducer of the CYP4 family of cytochromes P450 which does not, however, increase the total cytochrome

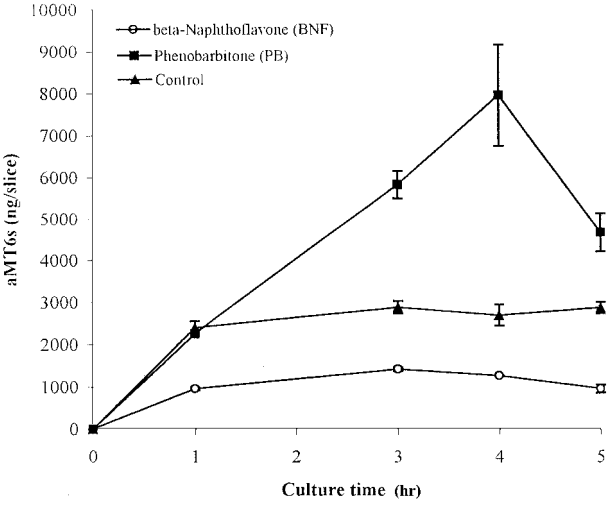

Fig. 9. Effect of phenobarbitone and β-naphthoflavone on the sulphate conjugation of 6-hydroxymelatonin by precision-cut liver slices. Precision-cut liver slices from animals pretreated with various cytochrome P450 inducers were incubated for various time periods, up to 5 hr, with 6-hydroxymelatonin (50 μM), and 6-sulphatoxymelatonin was determined in the medium. Results are presented as mean ± S.E.M. for three animals. Each culture time was carried out in duplicate and similarly each analysis was performed in duplicate.

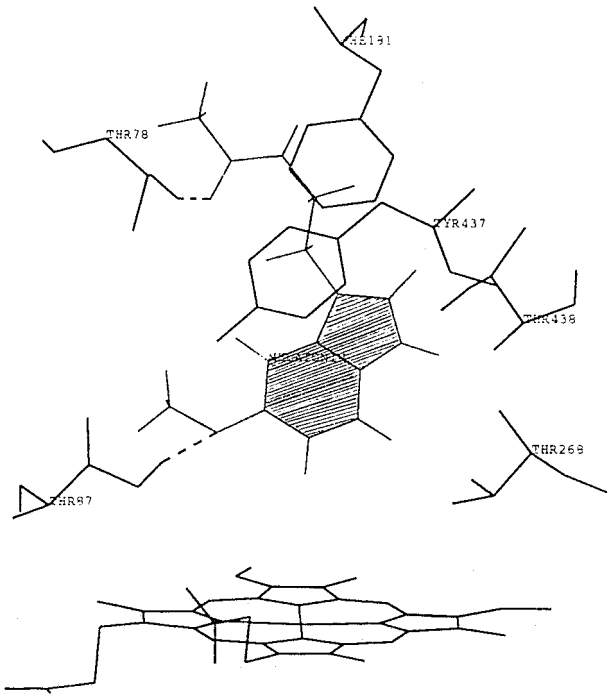

Fig. 10. Docking of melatonin within the human CYP1A2 putative active site. The melatonin structure was constructed using the Sybyl fragment library, and modelling was based on the indole nucleus. Melatonin is shown within the active site of CYP1A2 where two hydrogen bonds co-operatively position the molecule for 6-hydroxylation.

P450 concentration in liver microsomes, i.e. induction of CYP4 occurs at the expense of other families, in this case probably CYP1 [Lake and Lewis, 1996]; such a phenomenon has been extensively documented with many cytochrome P450 inducers [Safa et al., 1997]. In the studies utilizing liver slices, treatment with phenobarbitone led to a modest increase in the formation of 6-sulphatoxymelatonin, which could be ascribed to a rise in the sulphation of 6-hydroxymelatonin.

Additional studies on the effects of selective cytochrome P450 inhibitors and substrates on melatonin metabolism were undertaken for two reasons: (a) To evaluate the role of constitutive, non-inducible forms of cytochrome P450 in the 6-hydroxylation of melatonin, and (b) to establish whether both constituent enzymes of the CYP1A subfamily, namely 1A1 and 1A2, contribute to the hydroxylation of melatonin. Of all the inhibitors studied, α-naphthoflavone, an inhibitor of CYP1A1 and CYP1A2 [Murray and Reidy, 1990] and furafylline, a selective inhibitor of CYP1A2 [Pelkonen et al., 1998], caused a marked and concentration-dependent decrease in the generation of 6-sulphatoxymelatonin following incubation of melatonin with hepatic post-mitochondrial preparations. When 6-hydroxymelatonin replaced melatonin in the incubations, α-naphthoflavone, at the higher concentrations, caused a similar inhibition in the formation of 6-sulphatoxymelatonin. To our knowledge this is the first time that the inhibitory effect of α-naphthoflavone on sulphate conjugation has been reported. In recent studies [Ghazali and Waring, 1999], naturally occurring flavonoids were shown to inhibit the sulphation of p-nitrophenol in a non-competitive manner. Therefore, it appears that the inhibition of melatonin metabolism to the 6-sulphatoxy derivative by α-naphthoflavone reflects both, impairment of the sulphation pathway and of possibly cytochrome P450 metabolism. Furafylline, on the other hand, did not suppress the sulphate conjugation of 6-hydroxymelatonin, at the same concentrations, indicating that the marked reduction in the levels of 6-sulphatoxymelatonin following incubation with melatonin reflects entirely inhibition in the cytochrome P450-mediated hydroxylation of melatonin. However, caffeine, a selective substrate and inducer of CYP1A2 [Ayalogu et al., 1995], surprisingly failed to competitively inhibit the 6-hydroxylation of melatonin. Caffeine is rapidly metabolized by xanthine oxidase, a cytosolic enzyme, to a number of uric acid derivatives [Reinke et al., 1987]. Since in these studies post-mitochondrial preparations were used, it is likely that caffeine was rapidly metabolized, thus decreasing its effective concentration. Collectively, the induction studies showing an increase in the 6-hydroxylation of melatonin after treatment with β-naphthoflavone coupled with the marked inhibition in hydroxylation by furafylline provide strong evidence that CYP1A2 is an important catalyst in the hydroxylation of melatonin in rat liver microsomes. In a recent study [Yeleswaram et al., 1999], human cytochrome P450 proteins expressed in β-lymphoblastoid cell lines were incubated with melatonin. Of those studied, CYP1A1 and CYP1A2 where the most efficient in metabolizing melatonin. It should be emphasized that these authors measured disappearance of melatonin and not the formation of the 6-hydroxyderivative. It is also worth pointing out that, since CYP1A1 is very poorly expressed in the liver of control rats [Ioannides and Parke, 1990], CYP1A2 is the principal catalyst of the 6-hydroxylation of melatonin. The present findings may explain the interaction between fluvoxamine, a CYP1A2 inhibitor [Brøsen et al., 1993], and exogenous melatonin in humans, resulting in higher serum levels and bioavailability of the latter [Härtter et al., 2000]. Finally, at the highest concentration only, chlorzoxazone, a substrate of CYP2E1 [Jayyosi et al., 1995], also inhibited the metabolism of melatonin without concomitant effect in sulphation. This is likely a consequence of the fact that, at high concentrations, chlorzoxazone in the rat may be also metabolized by CYP1A proteins [Goasduff et al., 1995; Jayyosi et al., 1995]. It is pertinent to point out that the human CYP2E1, expressed in lymphoblastoid lines was unable to metabolize melatonin [Yeleswaram et al., 1999].

CYP1A2 substrates are planar [Lewis et al., 1986] characterized by a large area/depth^2 ratio [Lewis et al., 1987]. Melatonin, having an area/depth^2 ratio of 3.97 would be expected to serve as substrate of the CYP1 family. As the CYP1 family is highly conserved, the human orthologue has similar substrate specificity to the rat protein and share common amino acid sequences at the active site [Guengerich, 1997; Lewis et al., 1999]. Indeed, the computer modelling studies reported here demonstrate that melatonin is readily accommodated within the human CYP1A2 substrate-binding site in a position favouring 6-hydroxylation, the 6-position of melatonin being nearest to the haem. It would be thus anticipated that the human CYP1A2 will also catalyse the 6-hydroxylation of melatonin, and studies are now in progress to evaluate this.

In summary, we have demonstrated that CYP1A2 is a major catalyst of the 6-hydroxylation of melatonin in rat liver, at pharmacological concentrations, and it is likely to play a similar role in human liver. The physiological concentration of melatonin is orders of magnitude lower and the present findings cannot be extrapolated to the physiological situation. Melatonin metabolism may constitute a physiological function for CYP1A2, and the possibility that the pineal hormone melatonin may serve as substrate for this cytochrome P450 protein is an attractive one and supported by the observations that treatment with the CYP1A2 inhibitor, fluvoxamine, also increases the endogenous plasma levels of melatonin [Skene et al., 1994].

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