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Hepatic hydroxylation of melatonin in the rat is induced by phenobarbital and 7,12-dimethylbenz[a]anthracene – implications for cancer etiology

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Abstract. The protective function of the pineal hormone melatonin in the etiology of cancer and carcinogenic activation is increasingly well-established. Low melatonin levels seem to parallel cancer growth. The question arises as to which factors cause the depression of melatonin levels and what the direct effects are. Melatonin is known to be metabolized in the liver by hydroxylation and subsequent conjugation yielding 6-sulfatoxymelatonin as a main product. Nevertheless, the microsomal monoxygenases catalyzing the first step have been poorly investigated. To further characterize these enzymes, typical inducers of three different sub-classes, namely phenobarbital, 7,12-dimethylbenz[a]anthracene, and 17β-estradiol, were administered to female Fischer rats. Circadian urinary excretion patterns of melatonin and 6-sulfatoxymelatonin were determined over a 24-hour period on the third (second) day of induction. Liver homogenates were used to monitor the in vitro conversion of melatonin or 6-hydroxymelatonin to 6-sulfatoxymelatonin. Results of both approaches showed the microsomal monoxygenases catalyzing the 6-hydroxylation of melatonin to be strongly inducible by phenobarbital and to a lesser degree by the polyaromatic hydrocarbon 7,12-dimethylbenz[a]anthracene. The dramatic depletion of circulating melatonin as a result of these induction patterns and its possible implications for oncogenesis are discussed.
Key words. Dimethylbenz[a]anthracene; estradiol; phenobarbital; melatonin; 6-sulfatoxymelatonin; hepatic metabolism; urinary excretion; cancer etiology.

The concept that melatonin may have an important role in human and animal breast cancer is supported by various clinical and experimental studies. Lowered and modified urinary melatonin excretion is found in women suffering from breast cancer[1]. Patients with estrogen receptor-positive tumors show a depression of the nocturnal peak of melatonin[2]. In a consecutive study a progressive decline in the plasma peak of melatonin was observed during the growth of non-metastasized primary breast cancer[3]. On the other hand, exogenously administered melatonin inhibits experimental breast cancer induced by 7,12-dimethylbenz[a]anthracene (DMBA) in the female Sprague-Dawley rat, whereas pinealectomy equivalent to withdrawal of the main source of endogenous melatonin enhances tumor incidence[4,5].

Melatonin is metabolized in the liver by a two-step process consisting of oxidation by microsomal monoxygenases of the cytochrome P450 system, yielding 6-hydroxymelatonin (phase I-reaction), and subsequent conjugation to either 6-sulfatoxymelatonin by a cytosolic sulfotransferase (55–80%) or 6-glucuronylmelatonin by a membrane-bound glucuronyltransferase (5–30%)[6–8] (phase II-reaction). Additional metabolites like 5-methoxyindoleacetic acid[6], N-acetylserotonin[9], and cyclic 2-hydroxymelatonin[10], have been reported, but constitute a negligible proportion. Previous studies by Weinberg and Gasparini[11] showed that phenobarbital (PB) induces the 6-hydroxylation of melatonin by hepatic microsomal monoxygenases, but these enzymes have remained poorly characterized. In DMBA-treated rats a depression of nocturnal plasma melatonin was detected due to an enhanced or modified peripheral metabolism[12]. This finding gave further indication of the putative properties of the metabolizing enzymes responsible, and stressed the need for a more detailed characterization.

In order to investigate which sub-class of hepatic microsomal monoxygenases is involved in the 6-hydroxylation of melatonin, three typical inducers of different sub-classes, namely PB (PB-class), DMBA (class of polyaromatic hydrocarbons, PAH), and 17β-estradiol (E2, steroid-class), were administered to female Fischer rats to induce the hepatic degradation of the pineal hormone. To study this a combined approach was chosen consisting of 1) the determination of melatonin and 6-sulfatoxymelatonin in urine samples collected over a 24-hour period on the third (second) day of induction to monitor their circadian excretion patterns as well as possible phase shifts, and 2) the in vitro conversion of melatonin or 6-hydroxymelatonin to 6-sulfatoxymela-

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tonin in liver homogenates in order to discriminate between the combined phase I + II reaction and phase II reaction alone.

Materials and methods

Animals. Female Fischer F-344 rats were purchased (Charles River Wiga, Sulzfeld, Germany) at least 4 weeks prior to the experiments, to allow for acclimatization. The animals received a standard diet and water ad libitum and were kept under controlled environmental conditions (21.5 ± 0.5 °C, 55 ± 3% air humidity, white fluorescent light of usual intensity from 07.00 to 19.00 h, absolute darkness from 19.00 to 07.00 h German summertime).

Design of experiments. The different agents used for the induction of hepatic monoxygenases were administered intraperitoneally in established doses to groups of mature female Fischer F-344 rats aged 80 days (DMBA- and E2-experiment) or 115 ± 3 days (PB-experiment): PB at 8 mg/100 g body weight (b.wt) in saline [80 mg/ml] for three days in the morning[11], DMBA at 1.5 mg/100 g b.wt in peanut oil [15 mg/ml] for three days in the morning, and E2 at 0.1 mg/100 g b.wt in peanut oil [1 mg/ml] in the morning and in the evening on two successive days[13] (n = 12 in each experimental group and n = 12 in each age-matched control group receiving vehicle only at the same time as the experimental animals). On the second (E2 experiment) or third (PB or DMBA experiment) day all animals were transferred to metabolic cages (Tecniplast, Gazzada, Italy) and urine samples were collected as described below. After this the animals were killed during the day, livers were washed in ice-cold 0.1 M potassium phosphate buffer (pH 7.4), frozen in liquid nitrogen and stored at −25 °C, until in vitro conversions by liver homogenates of 6 individuals in each experimental and control group were studied.

Urine collection. The animals were placed in metabolic cages for a period of 24 h, starting from 07.00 h on the last day of drug administration. Water was given ad libitum, but food was withdrawn to improve the quality of liver microsomes prepared for the succeeding in vitro assays. Urine was collected at 4-hour intervals (with the exception of the 11.00–19.00 h interval in the PB-experiment), the volume of each collection was measured, and aliquots were stored at −25 °C until assayed for their melatonin and 6-sulfatoxymelatonin content. During the night collection tubes were changed under very dim red light of $\lambda > 665$ nm (filter RG 665 of Schott, Mainz, Germany, fitted on a dark chamber lamp).

In vitro assays. Frozen liver samples were quickly thawed, 2 g of liver of individual animals were homogenized manually in 6 ml ice-cold 0.1 M potassium phosphate buffer (pH 7.4), filtered through a piece of gauze, and centrifuged for ten minutes at 4 °C, 9000 × *g*. The supernatant containing microsomes (enzymes of phase I and partially phase II reaction) and cytosol (enzymes of phase II reaction, especially sulfotransferases) was used for the in vitro conversion of exogenous melatonin to 6-sulfatoxymelatonin (combined phase I + II reaction), as well as for the conversion of exogenous 6-hydroxymelatonin to 6-sulfatoxymelatonin (phase II reaction alone) according to a modification of the method described by Weinberg and Gasparini[11]. In detail, 1 ml reaction mixture consisting of 875 µl liver homogenate, 100 µl NADPH-regenerating system and 25 µl 2 mM melatonin solution in buffer, or 25 µl 2 mM 6-hydroxymelatonin, were incubated for one hour at 37 °C in an oxygen-saturated atmosphere. The reaction was stopped by the addition of 2.5 ml of methanol, which was also used to extract 6-sulfatoxymelatonin and to separate it from protein. After centrifuging for 15 min at 4 °C, 1600 × *g*, the precipitate was re-extracted with 1 ml of methanol and the combined methanol supernatants were dried under nitrogen. According to Weinberg and Gasparini[11] as well as Arendt et al.[14] this is the method recommended for the quantitative extraction of 6-sulfatoxymelatonin (> 90%). The residue was taken up in double-distilled water and was assayed for 6-sulfatoxymelatonin as described below.

The amount of endogenously formed 6-sulfatoxymelatonin was determined using the same reaction mixture as described above but with the addition of methanol to the reaction. Endogenous 6-sulfatoxymelatonin in liver homogenates was subtracted from the overall amount contained in homogenates after in vitro conversions to calculate the actual quantity of the metabolite formed. The protein content of the reaction mixture was measured according to Lowry et al.[15].

Quantification of melatonin and 6-sulfatoxymelatonin. Melatonin was quantified with RIA kits of WHB Bromma (Sweden), according to the method of Wetterberg et al.[16] without prior dilution of the samples. The intraassay coefficient of variation was 5% and the interassay coefficient of variation 8%. 6-Sulfatoxymelatonin was determined with RIA kits of Stockgrand (Guildford, UK) using an iodinated tracer of CIDtech (Mississauga, Canada), according to the method of Aldous and Arendt[17]. The cross-reactivities with related compounds were negligible at 0.5% for 6-hydroxymelatonin glucuronide and around 0.1% for 6-hydroxymelatonin[17]. To allow measurements within the linear range of the assay urine samples were diluted 1:50 to 1:500 and liver extracts 1:10 when endogenously formed 6-sulfatoxymelatonin was determined, and between 1:50 to 1:200 in the case of in vitro conversions of exogenous substrates. The intraassay coefficient of variation was 4% and the interassay coefficient of variation was 7%.

Evaluation. The arithmetic mean and the standard error of the mean (SEM) were calculated for each group and time-point. The statistical significance of group differences were analyzed by the nonparametric U-test of

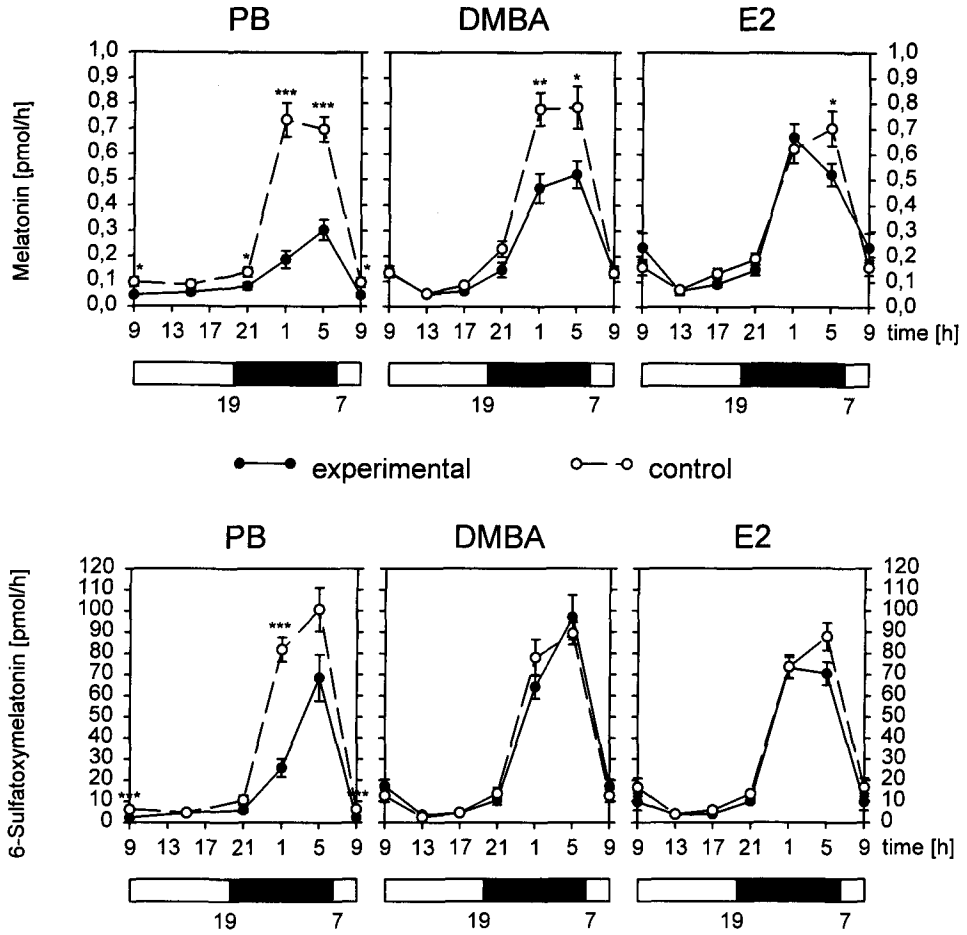

Figure 1. *Upper panel*: Average urinary melatonin excretion ± standard error of the mean (SEM) of female Fischer rats at different time-points over 24 hours. 12 rats in each group were treated with phenobarbital (PB), 7,12-dimethylbenz[a]anthracene (DMBA) or 17β-estradiol (E2) (closed circles, solid lines). Age-matched controls (n = 12 in each group) were treated with vehicle only (open circles, dashed lines). *Lower panel*: Average urinary 6-sulfatoxymelatonin excretion ± SEM of female Fischer rats at different time-points over 24 hours. Rats were treated as described for the upper panel.
White and black bars symbolize photoperiod of L:D = 12:12 (German summer time). *$0.05 \geq p > 0.01$; **$0.01 \geq p > 0.002$; ***$0.002 \geq p > 0.0001$ experimental vs. control group at certain time-points according to the U-test.

Wilcoxon, Mann and Whitney[18]. For the quantitative chronobiological evaluation of the circadian rhythms of melatonin and 6-sulfatoxymelatonin, the single cosinor analysis according to Nelson et al.[19] was performed. Three rhythm parameters were determined, namely the MESOR (i.e. arithmetic mean of the measured values within a 24-hour cycle), the amplitude (i.e. half of the rhythmic variability per 24 hours), and the acrophase (i.e. peak time of the cosine function used to approximate the rhythm).

Results

Urinary excretion of melatonin and 6-sulfatoxymelatonin. The average 24-hour urinary excretion patterns of melatonin and 6-sulfatoxymelatonin in the different groups are shown in figure 1. The most striking result is the dramatic decrease in melatonin excretion after phenobarbital-administration compared to age-matched controls. This decrease is found at all time intervals observed but particularly during the night, when a reduction of 75% ($p < 0.002$) and 57% ($p < 0.002$) occurs around 01.00 and 05.00 h, respectively. The decline of melatonin excretion is accompanied by a less pronounced drop in the excretion of 6-sulfatoxymelatonin (-68%, $p < 0.002$ around 01.00 h; -32%, $p < 0.1$ around 05.00 h). Administration of DMBA caused a moderate decrease in melatonin excretion, most noticeably around 01.00 h (-40%, $p < 0.01$) and 05.00 h (-34%, $p < 0.02$), whereas the excretion pattern of 6-sulfatoxymelatonin was nearly unchanged compared to controls. Administration of 17β-estradiol failed to cause major changes in melatonin or 6-sulfatoxymelatonin excretion patterns, but tended to shorten the duration of the nocturnal peak and produce a reduction in melatonin (-26%, $p < 0.05$) and 6-sulfatoxymelatonin (-20%, $p < 0.1$) excretion at 05.00 h.

Table. Results of the cosinor analysis for the urinary excretion of melatonin and 6-sulfatoxymelatonin [pmol/h] in Fischer rats after administration of different inducing agents or vehicle alone

Urinary excretion of	Group	n	Rhythm detection (p value)	MESOR ± SEM [pmol/h]	Amplitude [pmol/h]			Acrophase [h:min]	
					mean	range	% of MESOR	mean	range
Melatonin	PB (A)	12	$p < 0.0005$	0.124 ± 0.029	0.115	0.071–0.159	93	3:45	2:09–5:21
	control (B)	12	$p < 0.0005$	0.299 ± 0.054	0.374	0.293–0.455	126	2:49	1:56–3:44
6-Sulfatoxy-melatonin	PB (A)	12	$p < 0.0005$	19.60 ± 7.10	28.09	17.09–39.09	143	4:28	2:54–6:02
	control (B)	12	$p < 0.0005$	34.35 ± 7.65	51.53	40.62–62.44	150	3:18	2:22–4:14
Melatonin	DMBA (C)	12	$p < 0.0005$	0.230 ± 0.032	0.253	0.201–0.305	110	3:04	2:16–3:52
	control (D)	12	$p < 0.0005$	0.340 ± 0.048	0.420	0.346–0.494	124	2:38	1:58–3:18
6-Sulfatoxy-melatonin	DMBA (C)	12	$p < 0.0005$	32.83 ± 5.55	44.80	36.01–53.59	136	3:39	2:53–4:25
	control (D)	12	$p < 0.0005$	33.03 ± 5.18	46.94	38.70–55.18	142	3:04	2:22–3:46
Melatonin	E2 (E)	12	$p < 0.0005$	0.298 ± 0.039	0.303	0.248–0.358	102	3:21	2:29–4:13
	control (F)	12	$p < 0.0005$	0.320 ± 0.043	0.328	0.261–0.395	103	3:08	2:20–3:56
6-Sulfatoxy-melatonin	E2 (E)	12	$p < 0.0005$	32.53 ± 4.16	40.66	34.72–46.60	125	3:47	3:07–4:27
	control (F)	12	$p < 0.0005$	34.51 ± 4.72	44.74	37.31–52.17	130	3:29	2:50–4:10

n: number of animals, SEM: standard error of the mean; MESOR: rhythm adjusted 24-hour mean. MESOR test: urinary melatonin excretion: A vs B: $p < 0.0001$; C vs D: $p < 0.001$; urinary 6-sulfatoxymelatonin excretion: A vs B: $p < 0.01$. Amplitude test: urinary melatonin excretion: A vs B and C vs D: $p < 0.0001$; urinary 6-sulfatoxymelatonin excretion: A vs B: $p < 0.002$.

The results of the chronobiologic cosinor analysis are given in the table. All groups observed exhibited statistically significant circadian rhythms in melatonin and 6-sulfatoxymelatonin excretion ($p < 0.0005$) with comparable acrophases occurring between 02.30 and 04.30 h. Administration of PB led to a striking decrease in the melatonin MESOR (-59%, $p < 0.0001$) and amplitude (-69%, $p < 0.0001$), accompanied by a less pronounced drop in the 6-sulfatoxymelatonin MESOR (-43%, $p < 0.01$) and amplitude (-45%, $p < 0.002$). In DMBA-treated rats the melatonin MESOR was lowered by 32% ($p < 0.001$) and the melatonin amplitude by 40% ($p < 0.0001$), whereas the chronobiologic parameters of 6-sulfatoxymelatonin excretion were not affected by DMBA. Finally, the cosinor analysis did not reveal significant differences concerning melatonin and 6-sulfatoxymelatonin excretion after E2-treatment.

In vitro conversion of melatonin and 6-hydroxymelatonin to 6-sulfatoxymelatonin. Average values of the conversion of exogenous melatonin (upper panel) or 6-hydroxymelatonin (lower panel) to 6-sulfatoxymelatonin in liver homogenates of rats from the various groups are shown in figure 2. The results are expressed as per cent of controls. The administration of PB led to a tenfold increase in the degradation of melatonin to 6-sulfatoxymelatonin (phase I + II reaction) compared to controls ($p < 0.005$), whereas DMBA augmented this two-step reaction 4.6 fold ($p < 0.005$). Even in homogenates of E2-treated rats a 2.9 fold elevation could be found ($p < 0.025$).

Comparatively small changes were observed in the conjugation reaction from 6-hydroxymelatonin to 6-sulfatoxymelatonin showing a 35% depletion ($p < 0.01$) after DMBA and a marginal decrease after PB administration. E2 led to a statistically insignificant increase of 63%.

Discussion

The combined approach of determining melatonin and 6-sulfatoxymelatonin in urine and of monitoring in vitro conversion of melatonin and 6-hydroxymelatonin to 6-sulfatoxymelatonin by liver homogenates of Fischer rats treated with different types of hepatic monoxygenase inducers, leads to the conclusion that the hydroxylation of melatonin is strongly induced by phenobarbital and to a lesser degree by the polyaromatic hydrocarbon DMBA.

Rats treated with phenobarbital exhibited a dramatic decrease in urinary melatonin excretion at all time intervals observed, with a less pronounced drop in 6-sulfatoxymelatonin excretion. The resulting elevation of the ratio of 6-sulfatoxymelatonin/melatonin points to increased melatonin metabolism. A possible phase shift between melatonin and 6-sulfatoxymelatonin excretion can be ruled out by the results of the chronobiologic cosinor analysis showing comparable acrophases. In contrast, significantly lowered MESOR and amplitude were detected in the PB-treated group compared to controls. The data from the in vitro conversion strongly support the idea that enhanced metabolism is involved, revealing a tenfold increase in the degradation of melatonin to 6-sulfatoxymelatonin. Since the sulfation reaction does not seem to be affected by PB treatment, the enhanced conversion of melatonin to 6-sulfatoxymelatonin can only be due to an induction of the hydroxyla-

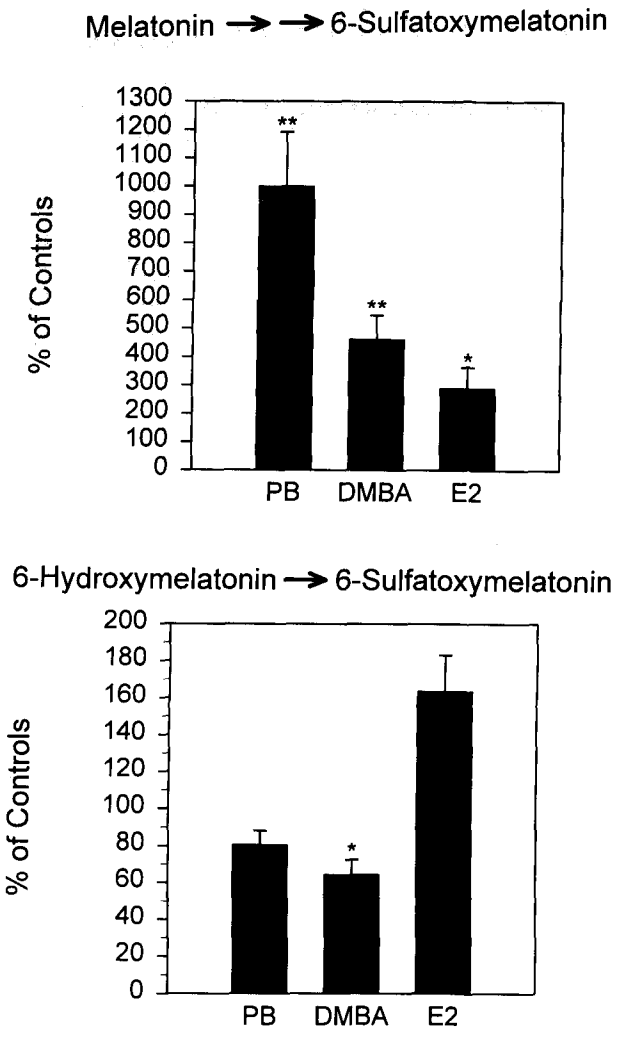

Figure 2. In vitro hepatic metabolism of melatonin. *Upper panel*: Specific activity (enzymatic activity/mg protein) of the combined phase I + II-reaction in liver homogenates of female Fischer rats treated with phenobarbital (PB), 7,12-dimethylbenz[a]anthracene (DMBA) or 17β-estradiol (E2). Average values $\pm$ standard error of the mean (SEM) of drug-treated groups (n = 6 in each group) expressed as % of age-matched controls (n = 6 in each group) treated with vehicle only, set as 100%. Absolute values of the controls were 1.08 fmol/mg × min (PB control), 1.02 fmol/mg × min (DMBA control) and 4.36 fmol/mg × min (E2 control). *Lower panel*: Specific activity of separate phase II-reaction in liver homogenates of female Fischer rats treated as described for the upper panel. Control values were 42.5 fmol/mg × min (PB control), 38.5 fmol/mg × min (DMBA control) and 60.5 fmol/mg × min (E2 control).
*$0.05 \geq p > 0.01$; **$0.01 \geq p > 0.002$ experimental vs. control group according to the U-test.

tion step, which confirms and extends the data of Weinberg and Gasparini[11].

The effect of PB on the 6-hydroxylation of melatonin can be summarized as follows: PB strongly induces the formation of 6-hydroxymelatonin leading to a depletion of circulating melatonin during the induction period. After two days of induction, this is reflected in a decreased urinary excretion of melatonin and, to a lesser extent, of 6-sulfatoxymelatonin.

After DMBA administration Fischer rats exhibit a moderate but distinct decrease in the circadian urinary melatonin excretion pattern, particularly at night, accompanied by a nearly unchanged excretion profile of 6-sulfatoxymelatonin. The resulting elevation of the 6-sulfatoxymelatonin/melatonin ratio again indicates an augmented melatonin metabolism. These data are in agreement with a previous study, in which a significant depression of the average nocturnal plasma melatonin concentration without corresponding alterations in 6-sulfatoxymelatonin was detected in Sprague-Dawley rats 2 and 7 days after the intragastral administration of DMBA[12]. In these findings a potential phase shift could not be ruled out, but the chronobiologic cosinor analysis of the current data allows us to exclude such a shift in the circadian rhythms of melatonin and 6-sulfatoxymelatonin. The results of the in vitro conversion of melatonin in liver homogenates also give clear evidence for an enhanced melatonin metabolism. The two-step conversion of melatonin to 6-sulfatoxymelatonin is amplified nearly fivefold. Interestingly, the second step of this metabolic route, namely the sulfation of 6-hydroxymelatonin to 6-sulfatoxymelatonin, is significantly reduced by 35%. An explanation for this phenomenon may be an increased formation of 6-hydroxymelatonin glucuronide. Verification of this hypothesis would require more refined analytical techniques, including HPLC and mass spectrometry, in order to determine all possible metabolites. However, a partial redirection of the metabolism of melatonin to 6-hydroxymelatonin glucuronide is very likely to happen since the genetic expression of UDP-glucuronyltransferase is under control of the same receptor (Ah) as the sub-class of microsomal monoxygenases induced by polyaromatic hydrocarbons such as DMBA[20].

The treatment of Fischer rats with 17β-estradiol did not result in marked differences in the pattern of melatonin or 6-sulfatoxymelatonin excretion, although the nocturnal peak appeared to have been shortened. This may be due to negative feedback of 17β-estradiol on the pineal organ. It has been known for a long time that melatonin not only exerts inhibitory effects on the gonads, but gonadal steroids also negatively affect the secretion of pineal melatonin. At proestrus when the circulating levels of 17β-estradiol are high, urinary melatonin excretion in the rat is significantly lowered due to decreased production[21]. In comparison with the extraordinary influence of phenobarbital and DMBA upon melatonin degradation and excretion the effects of 17β-estradiol are, however, only marginal, although the in vitro metabolism of melatonin in liver homogenates of 17β-estradiol-treated rats is also slightly elevated.

The question arises as to which isoenzyme(s) of the cytochrome P450 system may be involved in the metabolism of melatonin. Since phenobarbital and PAHs such as DMBA are known to induce separate

sets of P450 isoenzymes[22,23], it appears very likely that the observed pronounced enhancement of the oxidative metabolism of melatonin after treatment with PB and DMBA can be attributed to different isoenzymes, which, however, seem to possess a common substrate specificity for melatonin. The present findings will now be discussed according to the current nomenclature of cytochrome P450 enzymes[24]. The following rat isoenzymes have been reported to be inducible by PB: CYP2B1, CYP2B2, CYP2C6, CYP3A1 and CYP3A2[22,25,26]. CYP2B1 and CYP2B2 exhibit similar broad, overlapping specificities for a number of substrates, e.g. benzphetamine (N-demethylation), hexobarbital (3-hydroxylation) and zoxazolamine (6-hydroxylation). For the vast majority of substrates, the activity of 2B1 is much greater than that of 2B2[25]. CYP2C6 catalyzes various hydroxylation reactions of substrates such as hexobarbital, zoxazolamine, 17β-estradiol, acetanilide and R-warfarin, but in most cases metabolism occurs at comparatively low rates[25]. The 6β-hydroxylation of steroids and various other hydroxylation and demethylation reactions are carried out by CYP3A1 and CYP3A2, the latter being a male-specific enzyme[23,25]. Since PB-induction enhanced the metabolism of melatonin to 6-sulfatoxymelatonin in female rats very efficiently, the isoenzymes CYP2B1, CYP2C6 or CYP3A1 would be the potential catalysts involved in the degradation of melatonin.

PAHs analogous to DMBA are known to induce the isoenzymes CYP1A1, CYP1A2, CYP2A1 and CYP-2A3[23]. CYP1A1 is involved in the metabolism of PAHs such as benzo[a]pyrene, whereas CYP1A2 has a high level of catalytic activity for arylamines and can activate several heterocyclic amine promutagens derived from pyrolysates of proteins. Both isoenzymes have overlapping specificities and low levels of catalytic activity for other substrates (e.g. 7-ethoxyresorufin[23]). CYP2A1 has been shown to specifically hydroxylate testosterone at the 7α position and also to a lesser extent at the 6α position[23]. Since CYP2A3 is expressed exclusively in rat lung[27], CYP1A2 seems to be most probably involved in the metabolism of melatonin within the group of PAH-inducible P450 isoenzymes. It cannot be excluded that the 6-hydroxylation of melatonin is carried out by (an)other not yet identified P450 isoenzyme(s), which may even show overlapping inducibility by PB and DMBA.

Another relevant question is, what are the possible consequences of dramatically decreased levels of circulating melatonin after treatment with inducers such as PB and DMBA? In recent years melatonin has been considered to be involved in antioxidative processes and was found to be one of the most potent naturally occurring radical scavengers[28–31]. The extremely high diffusibility of the melatonin molecule allows it to enter all cells of the body and every subcellular compartment. It can be detected in the cell nuclei of most mammalian tissues, where it could interact with nuclear proteins[32]. Within the nucleus melatonin may exert one of its most important anti-cancer effects by scavenging reactive oxygen species and electrophilic intermediates which directly modify and damage DNA and are known to be generated by inorganic and organic carcinogens such as $KBrO_3$ and polyaromatic hydrocarbons including DMBA[33]. Since the radical-scavenging function of melatonin was shown to be strictly dose-dependent in the safrole system[34], a decreased melatonin concentration may be directly connected with a diminished protection of the DNA, leading to a higher cancer risk. Exposure of an organism to carcinogens like DMBA therefore not only confronts it with a potential DNA-damaging agent, but also consumes the DNA-protective molecule melatonin by enhancing its metabolism in the liver. The situation is further complicated by the fact that exogenously administered melatonin also modulates the activity of phase I and II drug metabolizing enzymes in normal as well as DMBA-treated rats[35]. The phase I-enzymes cytochrome P450 and cytochrome b_5 that lead to the carcinogenic activation of DMBA can be significantly inhibited by melatonin, whereas the detoxifying phase II enzyme glutathione S-transferase and its cofactor glutathione are significantly induced. This means that high melatonin levels can prevent the formation of dangerous DMBA metabolites, whereas reduced levels of melatonin result in an enhanced metabolic activation of the carcinogen as well as in a diminished detoxification via conjugation or radical scavenging. Since circulating melatonin can be dramatically reduced by different chemical compounds such as phenobarbital and the polyaromatic hydrocarbons DMBA and 3-methylcholanthrene[36] it can be assumed that individuals exposed to combinations of such substances have a reduced capacity to protect themselves against carcinogenic threats.

In future it will be interesting to find out whether exogenously administered melatonin is able to compensate for the metabolic depression of melatonin and to enhance this natural protection against cancer.

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1 Bartsch, C., Bartsch, H., Jain, A. K., Laumas, K. R., and Wetterberg, L., J. neural Transm. *52* (1981) 281.
2 Tamarkin, L., Danforth, D., Lichter, A., DeMoss, E., Cohen, M., Chabner, B., and Lippman, M., Science *216* (1982) 1003.
3 Bartsch, C., Bartsch, H., Fuchs, U., Lippert, T. H., Bellmann, O., and Gupta, D., Cancer *64* (1989) 426.
4 Tamarkin, L., Cohen, M., Roselle, D., Reichert, C., Lippman, M., and Chabner, B., Cancer Res. *41* (1981) 4432.
5 Aubert, C., Janiaud, P., and Lecalvez, J., J. neural Transm. *47* (1980) 121.
6 Kopin, I. J., Pare, C. M. B., Axelrod, J., and Weissbach, H., J. biol. Chem. *236* (1961) 3072.
7 Kveder, S., and Mclsaac, W. M., J. biol. Chem. *236* (1961) 3214.

8 Jones, R. L., McGeer, P. L., and Greiner, A. C., Clinica. chim. Acta *26* (1969) 281.
9 Young, I. M., Leone, R. M., Francis, P., Stovell, P., and Silman, R. E., J. clin. Endocr. Metab. *60* (1985) 114.
10 Vakkuri, O., Tervo, J., Luttinen, R., Ruotsalainen, H., Rahkamaa, E., and Leppäluoto, J., Endocrinology *120* (1987) 2453.
11 Weinberg, U., and Gasparini, F. J., in: Melatonin Rhythm Generating System. Int Symp, Bethesda, MD, 1981, p. 193. Ed. D. C. Klein. Karger, Basel 1982.
12 Bartsch, C., Bartsch, H., Lippert, T. H., and Gupta, D., Neuroendocrinology *52* (1990) 538.
13 Breskvar, K., Steroids *48* (1986) 85.
14 Arendt, J., Bojkowski, C., Franey, C., Wright, J., and Marks, V., J. clin. Endocr. Metab. *60* (1985) 1166.
15 Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. biol. Chem. *193* (1951) 265.
16 Wetterberg, L., Eriksson, O., Friberg, Y., and Vangbo, B., Clinica chim. Acta *86* (1978) 169.
17 Aldous, M. E., and Arendt, J., Ann. clin. Biochem. *25* (1988) 298.
18 Sachs, L., Applied Statistics: A Handbook of Techniques. Springer, New York 1984.
19 Nelson, W., Tong. Y. L., Lee, J.-K., and Halberg, F., Chronobiologia *6* (1979) 305.
20 Nebert, D. W., and Gonzalez, F. J., A. Rev. Biochem. *56* (1987) 945.
21 Ozaki, Y., Wurtman, R. J., Alonso, R., and Lynch, H. J., Proc. natl Acad. Sci. USA *75* (1978) 531.
22 Okey, A. B., Pharmac. Ther. *45* (1990) 241.
23 Gonzalez, F. J., Pharmac. Ther. *45* (1990) 1.
24 Nelson, D. R., Kamataki, T., Waxman, D. J., Guengerich, F. P., Estabrook, R. W., Feyereisen, R., Gonzalez, F. J., Coon, M. J., Gunsalus, I. C., Gotoh, O., Okuda, K., and Nebert, D. W., DNA Cell Biol. *12* (1993) 1.
25 Ryan, D. E., and Levin, W., Pharmac. Ther. *45* (1990) 153.
26 Wrighton, S. A., and Stevens, J. C., Crit. Rev. Toxic. *22* (1992) 1.
27 Kimura, S., Kozak, C. A., and Gonzalez, F. J., Biochemistry *28* (1989) 3798.
28 Ianăş, O., Olinescu, R., and Bădescu, I., Rom. J. Endocr. *29* (1991) 147.
29 Poeggeler, B., Reiter, R. J., Tan, D.-X., Chen, L.-D., and Manchester, L. C., J. pineal Res. *14* (1993) 151.
30 Reiter, R. J., Poeggeler, B., Tan, D.-X., Chen, L.-D., Manchester, L. C., and Guerrero, J. M., Neuroendocr. Lett. *15* (1993) 103.
31 Reiter, R. J., Braz. J. med. biol. Res. *26* (1993) 1141.
32 Menendez-Pelaez, A., Poeggeler, B., Reiter, R. J., Barlow-Walden, L., Pablos, M. I., and Tan, D.-X., J. cell. Biochem. *53* (1993) 373.
33 Frenkel, K., Pharmac. Ther. *53* (1992) 127.
34 Tan, D.-X., Poeggeler, B., Reiter, R. J., Chen, L.-D., Chen, S., Manchester, L. C., and Barlow-Walden, L. R., Cancer Lett. *70* (1993) 65.
35 Kothari, L., and Subramanian, A., Anti-Cancer Drugs *3* (1992) 623.
36 Bartsch, C., Praast, G., Peters, C., Bartsch, H., Mecke, D., and Lippert, T. H., in: Melatonin and the Pineal Gland – From Basic Science to Clinical Application, p. 317. Eds Y. Touitou, J. Arendt and P. Pévet. Elsevier Science Publishers B.V., Amsterdam 1993.