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Scientific Correspondence

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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From: Dr. Y.-J. Surh, Assistant Professor of Biochemical and Molecular Toxicology, Yale University School of Medicine, Department of Epidemiology and Public Health, New Haven (Connecticut 06520-8034, USA)

15 May 1995

Sir,

Praast and colleagues have reported that hepatic microsomal monooxygenase activity catalyzing the melatonin hydroxylation is strongly inducible by phenobarbital[1]. The authors state that the resulting metabolite, 6-hydroxymelatonin undergoes phase II (conjugation) reactions such as glucuronidation and sulfation. In this study, urinary excretion of the sulfo-conjugated metabolite of 6-hydroxymelatonin was measured. Furthermore, the formation of 6-sulfooxymelatonin was examined by incubating melatonin or its 6-hydroxylated metabolite with rat liver postmitochondrial supernatant (S-9) enriched with the NADPH-regenerating system. Unfortunately, part of experimental procedures described in this paper are confusing and misleading. I have no objection to hydroxylation of melatonin by rat liver S-9 and NADPH. However, sulfonation of resulting 6-hydroxymelatonin does not appear to have been assessed in a correct manner because the reaction was conducted without the biological sulfo-group donor or co-substrate, 3′-phosphoadenosine-5′-phosphosulfate (PAPS). Since PAPS was not added to the incubation mixture, the sulfotransferase-mediated formation of 6-sulfooxymelatonin from melatonin via 6-hydroxymelatonin or directly from the hydroxy substrate must have been solely dependent upon the presence of endogenous PAPS, and it is unlikely that the sulfonation occurred to a significant extent. Even though the radio-immuno assay employed in this study might be sensitive enough to detect the basal level of 6-sulfooxymelatonin, the amount of this polar metabolite is considered to have been underestimated because of limitations of the sulfo-group donor required for its formation by sulfotransferase activity. Addition of the NADPH-generating system was intended to facilitate the microsomal monooxygenase-mediated metabolism of melatonin to its 6-hydroxy derivative. However, the same cofactor system was also used for the determination of sulfonation of 6-hydroxymelatonin. This does not make any sense because the hydroxy derivative already bears a functional group that can be subjected to sulfo-conjugation. The presence of NADPH, on the contrary, would only complicate the reaction by producing secondary metabolite(s) through oxidation of 6-hydroxymelatonin, limiting its availability for the formation of 6-sulfooxymelatonin. Thus, due to omission of the sulfo-group donor and the inappropriate use of the NADPH-generating system, the accurate and precise quantitation of 6-sulfooxymelatonin from melatonin or 6-hydroxymelatonin could not be expected and the significance of in vitro formation of 6-sulfooxymelatonin addressed by the authors thus needs to be reconsidered. Finally, I would like to suggest that the term 'sulfonation' be used in place of 'sulfation' since sulfotransferases catalyze the transfer of the sulfo-group ($-SO_3^-$), not the sulfate ($-SO_4^{2-}$), from PAPS to the hydroxyl or amino groups of acceptor molecules[2]. By analogy, the use of the prefix 'sulfooxy-' or 'sulfonoxy-' is a more appropriate nomenclature of designating *sulfonated* products, including the sulfo conjugate of 6-hydroxymelatonin, than the use of the unfamilar term 'sulfatoxy-' adopted by the authors.

1 Praast, G., Bartsch, C., Bartsch, D., Mecke, D., and Lippert, T. H., Experientia *51* (1995) 349.
2 Miller, J. A., and Surh, Y.-J. in: Conjugation-Deconjugation Reactions in Drug Metabolism and Toxicity (Handbook Exp. Pharmacol., vol. 112), p. 429. Ed F. C. Kauffman, Springer-Verlag, Heidelberg 1994.

Young-Joon Surh

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Response from: Dr. Dipl. Biochem. C. Bartsch, Research Scientist, Section of Clinical Pharmacology, University Women's Hospital, D-72076 Tübingen (Germany)

29 June 1995

Sir,

We are writing in response to the letter of Dr. Surh concerning our paper recently published in your journal[1].

The aim of this study was to investigate whether the hepatic hydroxylation of melatonin to 6-hydroxymelatonin is enhanced by the administration of the inducing agents 7,12-dimethylbenz[a]anthracene (DMBA), phenobarbital (PB) or 17ß-estradiol (E2) leading to a reduction of circulating melatonin. In our experiments we quantitated the in vitro enzymatic activities of the combined phase I- and phase II-reaction of the degradation of melatonin to 6-sulfatoxymelatonin (aMT6s) via 6-hydroxymelatonin. This appraoch had to be chosen because no highly sensitive HPLC with fluorescence detection for 6-hydroxymelatonin, the product of phase I-reaction, was available at that time for us. In a separate experiment we measured the formation of aMT6s from 6-hydroxymelatonin as a result of phase II-reaction. Phase I + II- as well as phase II-reaction were performed under comparable in vitro conditions using the same concentrations of substrates to allow for comparison of the experimental results achieved.

In his letter, Dr. Surh expressed his concern that the experimental conditions chosen by us may not have given correct results, since no exogenous PAPS was added to supplement the limited endogenous sulfate pool which would negatively affect the quantitative metabolic degradation of melatonin to aMT6s. From the lower panel of figure 2 of our paper depicting the conversion from 6-hydroxymelatonin to aMT6S (phase II-reaction) it is in fact obvious that this enzymatic reaction is reduced after the administration of PB and DMBA, perhaps due to either a depletion of endogenous sulfate under the influence of an accelerated metabolic degradation or an increased formation of glucuronide as discussed in the paper. From this observation, however, it is premature to conclude that the combined phase I + II reaction serving as an indirect measure for the rate of phase I-reaction (i.e. the conversion of melatonin to 6-hydroxymelatonin) is also limited by the availability of endogenous sulfate. This conclusion would only hold if phase II-reaction was the rate-limiting step of the conversion of melatonin to aMT6s. The absolute values of the specific enzymatic activities of phase I + II- as well as phase II-reaction of the control groups given in the legend of figure 2 show that phase II-reactions occur at a 15–40-fold higher rate than phase I + II reactions. Even after administration of PB, the strongest inducing agent tested, when a tenfold acceleration of this reaction is observed, phase II-reaction takes place at a 3-fold higher rate than phase I + II-reaction, indicating that phase I still remains the rate-limiting step. It can therefore be concluded that the combined phase I + II-reaction without the addition of exogenous PAPS gives evidence for an induction of the phase I-reaction after treatment by PB, DMBA or 17ß-estradiol in our experiments. An actually enhanced formation of 6-hydroxymelatonin was confirmed by us in a subsequent study measuring the hepatic content of 6-hydroxymelatonin after PB- and DMBA-administration using HPLC with fluorescence detection and subsequent mass spectrometry[2].

Another point of criticism of Dr. Surh concerned the addition of a NADPH-regenerating system to the in vitro conversion of 6-hydroxymelatonin to aMT6s (phase II-reaction) where it was actually not required functionally. Our rationale to add the regenerating system to this reaction as well was to allow direct comparison between the tests of phase II-reaction and the combined phase I + II -reaction for which NADPH was required. We do not think, as assumed by Dr. Surh, that addition of the NADPH-regenerating system leads to a substantial distortion of our results due to the formation of undesired side-products during phase II-reaction. If, however, such products were formed they would also be generated during the combined phase I + II-reaction used to estimate phase I-reaction. Therefore it was necessary to see in which way the separate phase II-reaction was affected by the addition of the NADPH-regenerating system, as was done in our tests.

With reference to the suggestion of Dr. Surh to rather term the conversion of 6-hydroxymelatonin to aMT6s 'sulfonation' rather than 'sulfation', we would like to mention that 'sulfation' is, at present, used as the predominant terminology in the field of pharmacology and toxicology and therefore aMT6s is generally termed 6-sulfatoxymelatonin. This view is supported also by Weinsilboum and Otterness in their chapter on 'Sulfotransferse Enzymes'[3] which appeared in the same volume of the *Handbook of Experimental Pharmacology* as the chapter by Miller and Surh entitled 'Sulfonation in Chemical Carcinogenesis'[4]. We cannot judge which terminology, on the basis of the actual chemical reaction mechanisms involved, will prove to be the appropriate one for future use.

Christian Bartsch

1 Praast, P., Bartsch, C., Bartsch, H., Mecke, D., and Lippert, T. H., Experientia *51* (1995) 349.
2 Bartsch, C., Praast, G., Peter, 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.
3 Weinsilboum, R., and Otterness, D., in: Conjugation-Deconjugation Reactions in Drug Metabolism and Toxicity (Handbook Exp. Pharmacol., vol. 112), p. 45. Ed. F. C. Kauffman. Springer-Verlag, Heidelberg 1994.
4 Miller, J. A., and Surh, Y.-J., in: Conjugation-Deconjugation Reactions in Drug Metabolism and Toxicity (Handbook Exp. Pharmacol., vol. 112), p. 429. Ed. F. C. Kauffman. Springer-Verlag, Heidelberg 1994.