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The selective activation of cytochrome P-450 dependent microsomal hydroxylases in human and rat liver microsomes

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The synthetic steroid betamethasone has been shown to cause a rapid and selective activation of biphenyl 2-hydroxylation and benzpyrene 3-hydroxylation when added to rat liver microsomes from male rats which are fortified with NADPH [1, 2]. Many other cytochrome P-450 dependent reactions are unaffected by the presence of this steroid, e.g. ethoxycoumarin deethylation, ethoxy resorufin deethylation, aniline 4-hydroxylation [3]. Activation *in vivo* in rats in biphenyl 2-hydroxylation by betamethasone has also been demonstrated [4].

The present study was undertaken in order to ascertain (a) what other hydroxylation reactions can be activated by betamethasone (b) whether activation can occur in human liver.

Materials and Methods

Human liver samples were obtained from either renal transplant donors who had met traumatic death and were maintained on life support systems until the kidneys were removed or by wedge biopsy at laparotomy where histology was required. Tissue surplus to histological requirement was made available for these studies. The local Research Ethics Committee and, where necessary, the appropriate Coroner's permission were obtained to use such material in these studies. Samples used were histologically normal or had slight non-specific changes. Patients were not receiving any chronic drug therapy but some did receive general anaesthesia or such drugs as were necessary during maintenance on life support systems.

Microsomes were prepared from rat [4] or human [5] livers as described previously. Incubations with biphenyl were performed as described previously [4] and were terminated after 5 min with rat liver and after 10 min with human liver. Hydroxybiphenyls were determined by solvent extraction followed by h.p.l.c. [6]. Antipyrine incubations contained 0.825 μCi [3-^{14}C]antipyrine (sp. act. 5 μCi/μmole synthesised from [3-^{14}C]ethylacetoacetate and phenylhydrazine followed by methylation), 6 mM $MgCl_2$, 50 mM Tris–HCl buffer (pH 7.4), cold antipyrine to a final concentration of 20 mM antipyrine and 1 or 2 mg microsomal protein/ml for rat and human liver respectively. Reactions were started by addition of 1.2 mM NADPH giving a final volume of 250 μl. Betamethasone (10^{-4} M) was added in dimethylformamide so that the concentration of dimethylformamide was 0.5% in biphenyl incubations and 1.0% in antipyrine incubations. Incubations with antipyrine were terminated after 12 min and the metabolites were assayed by reverse phase h.p.l.c. and liquid scintillation spectrometry [7].

Results and discussion

Dimethylformamide (0.5–1.0%, final concentration) had little effect on biphenyl or antipyrine metabolism in either rat or human liver microsomes. Although the basal levels of hydroxylation activity at the 4- and 3-methyl positions of antipyrine and the 2-position of biphenyl are considerably greater in rat liver than in human liver the rates of hydroxylation of biphenyl in the 4- and 3-positions are similar in the two species. These observations demonstrate that the commonly held assumption that mixed function oxidase activity (expressed on a per mg protein basis) is invariably lower in man than rodents [8] is incorrect. Betamethasone significantly activated biphenyl 2-hydroxylation but not biphenyl 3- and 4-hydroxylation in rat liver microsomes. Betamethasone also caused a significant increase in rat liver microsomal antipyrine 4-hydroxylation but not in the formation of 3-hydroxymethylantipyrine (see Table 1). Similar results were obtained in human liver

Table 1. Selective activation of microsomal antipyrine 4-hydroxylation and biphenyl 2-hydroxylation in rat and human liver

Species	Betamethasone (M)	*n*	Antipyrine 3-Methyl hydroxylation	Antipyrine 4-Hydroxylation
Rat	0	6	3.59 ± 0.32*	1.59 ± 0.18
	10^{-4}	6	94.9% ± 3.5†	214% ± 21.1
Human	0	6	0.53 ± 0.13*	0.40 ± 0.11
	10^{-4}	6	98.2% ± 6.7†	163.7% ± 15.6
			Biphenyl 2-Hydroxylation	Biphenyl 3-Hydroxylation	Biphenyl 4-Hydroxylation
Rat	0	65	0.25 ± 0.02*	0.09 ± 0.01	0.78 ± 0.03
	10^{-4}	65	430% ± 24†	101% ± 3	101% ± 2
Human	0	4	0.007 ± 0.001*	0.138 ± 0.022	0.97 ± 0.025
	10^{-4}	4	336% ± 101†	97% ± 4	95% ± 4

Results are means of *n* different livers ± S.E.M.

* Control activities (containing 0.5–1.0% dimethylformamide) are expressed as nmoles/min/mg of microsomal protein.

† Activities in the presence of betamethasone are expressed as percentage of control activity.

microsomes; activation of biphenyl 2-hydroxylation was very marked, activation of antipyrine 4-hydroxylation although significant was less pronounced. As in the rat betamethasone had no significant effect on biphenyl 3- and 4-hydroxylation or formation of 3-hydroxymethyl-antipyrine. Although the overall effects of betamethasone on biphenyl 2-hydroxylation and antipyrine 4-hydroxylation indicated that a similar mechanism was involved in the activations, for individual livers the activation of these two reactions was not always in parallel. Thus human liver 4 (43 years old male caucasian kidney donor) showed a significant increase in antipyrine 4-hydroxylation to 183 per cent of the control value but a rather small enhancement of biphenyl 2-hydroxylation to 150 per cent, whereas human liver 3 (7 year old male caucasian kidney donor) was enhanced to only 112 per cent for antipyrine 4-hydroxylation but was increased to 675 per cent for biphenyl 2-hydroxylation. These data imply that the two hydroxylation reactions activated by betamethasone are carried out by very similar but not identical enzyme systems. Interestingly the sex difference in activation which is observed in rats [2] does not occur in human liver for the adult female liver in the group we studied gave very similar activation of biphenyl-2-hydroxylation to the male human livers. It has recently been shown [3, 9, 10] that α-naphthoflavone activates a similar but not identical profile of P-450 dependent reactions to that activated by betamethasone. Thus the picture is emerging of a number of P-450 dependent enzyme activities which are normally partially or totally latent in the endoplasmic reticulum but which are selectively and rapidly activatable by several different types of endogenous and exogenous chemicals. It may be significant in this regard that the structural requirements for both betamethasone and naphthoflavone type activations appear to be rather precise [4, 9]. The fact that in the present report we have demonstrated that activation occurs in human liver microsomes and have previously shown it to occur in intact rat hepatocytes and rats *in vivo* indicates the likelihood that a similar activation may occur in man *in vivo*. The physiological relevance of this rapid and selective activation of certain forms of P-450 remains to be established.

Summary

The activity of P-450 dependent hydroxylation of antipyrine in the 4- and 3-methyl- positions of antipyrine and the 2-position of biphenyl is considerably lower in human rat liver microsomes whereas for the 3- and 4-hydroxylation of biphenyl, the activities are very similar. Addition of betamethasone to the microsomes selectively activates biphenyl 2-hydroxylation and antipyrine-4-hydroxylation in both rat and man. Considerable interindividual differences in the magnitude of activation are observed in man. The biological significance of this activation phenomenon is discussed.

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REFERENCES

1. J. M. Tredger, F. J. McPherson, J. Chakraborty, J. W. Bridges and D. V. Parke, *Archs. Pharmac.* **292**, 267 (1976).
2. D. J. Benford and J. W. Bridges, *Biochem. Soc. Trans.* **7**, 1107 (1979).
3. D. J. Benford and J. W. Bridges, *Biochem. Pharmac.* **29**, 2345 (1980).
4. D. J. Benford, J. W. Bridges and D. V. Parke, *Xenobiotica* **10**, 329 (1980).
5. A. R. Boobis, M. J. Brodie, G. C. Kahn, D. R. Fletcher, J. H. Saunders and D. S. Davies, *Br. J. Clin. Pharmac.* **9**, 11 (1980).
6. M. D. Burke, D. J. Benford, J. W. Bridges and D. V. Parke, *Biochem. Soc. Trans.* **5**, 1370 (1977).
7. G. C. Kahn, A. R. Boobis, I. A. Blair, M. J. Brodie and D. S. Davies, *Analyt. Biochem*, in press.
8. C. H. Walker, *Drug Metab. Rev.* **7**, 295 (1980).
9. F. J. Wiebel, J. C. Leutz, L. Diamond and H. V. Gelboin, *Archs. Biochem. Biophys.* **144**, 78 (1971).
10. D. J. Benford, Ph.D. Thesis, University of Surrey, (1980).