Biochemical Pharmacology, Vol. 36, No. 14, pp. 2279–2282, 1987.
Printed in Great Britain.

0006-2952/87 $3.00 + 0.00
Pergamon Journals Ltd.

GENETIC VARIATION IN CYTOCHROME P-450-DEPENDENT DEMETHYLATION IN *DROSOPHILA MELANOGASTER*

INGER HÄLLSTRÖM

Department of Toxicological Genetics, Wallenberg Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden

(*Received* 9 *September* 1985; *accepted* 23 *December* 1986)

Abstract—The genetic variation in the basal capacity to *N*-demethylate aminopyrine, *d*-benzphetamine and ethylmorphine was studied in microsomes from adult *Drosophila* of 9 different strains. Ethylmorphine and *d*-benzphetamine *N*-demethylase activity varied about fourfold between the strains, with the highest capacity for both reactions in the Aflatoxin B_1-sensitive Florida 9 and the lowest in the insecticide-resistant Hikone R. The two activities were closely correlated with each other but not with aminopyrine demethylation or any previously studied cytochrome P-450-dependent reaction, indicating a common determination by a separate cytochrome P-450 form(s). Aminopyrine *N*-demethylase activity was more than fourfold higher in the DDT-resistant Oregon R than in Berlin K. A genetic analysis of aminopyrine *N*-demethylation revealed that the high activity in the Oregon R(R) strain was inherited as an apparently semidominant second chromosome trait. The similar mode of inheritance as well as the close correlation between aminopyrine demethylase and the previously analysed biphenyl 4-hydroxylase activity suggests that these activities are under the same genetic control.

The fruit fly, *Drosophila melanogaster*, is widely used as a test organism in the screening for mutagenic and carcinogenic effects of chemicals [1–3]. It represents a unique system between bacterial and *in vitro* systems, and the intact mammalian tests. With *Drosophila*, it is possible to analyse the effects of treatment of an intact, eukaryotic organism with relevant dose ranges, to get information concerning the complex and balanced *in vivo* situation. This approach is seriously restricted in mammalian systems for practical and economical reasons. It has been demonstrated that *Drosophila* has the capacity to bioactivate most classes of indirect carcinogens [1–3]. The cytochrome P-450 enzyme system in *Drosophila melanogaster* has been shown to be inducible, to consist of several isozyme forms and to have the capacity to metabolize a variety of substrates [4–8], thus resembling the corresponding mammalian system. Quantitative and qualitative differences do, however, occur, particularly in the metabolism of, and induction by, polycyclic aromatic hydrocarbons [6, 9–11]. Thus, a further characterisation of the capacity and limitation of the *Drosophila* cytochrome P-450 enzyme system is of importance for the reliability of *Drosophila* test systems for the detection of indirect carcinogens and mutagens. A substantial genetic variation in the capacity to metabolize different substrates and in the pattern of specific protein bands, demonstrated by SDS-polyacrylamide gel electrophoresis of the microsomal fractions, has also been observed between *Drosophila* strains of different geographic origin and history of xenobiotic exposure [10, 12–13]. Such variation has impact on the choice of appropriate test strains. Four to five genes determining different cytochrome P-450-dependent activities in *Drosophila*, located at different places on the second and third chromosomes, have been demonstrated [14–16]. In the present study, the characterization of the cytochrome P-450 system in *Drosophila* is extended to study the genetic variation in the capacity of uninduced flies to *N*-demethylate aminopyrine, ethylmorphine and *d*-benzphetamine.

MATERIALS AND METHODS

Aminopyrine and ethylmorphine (analytical grade) was purchased from Karolinska Hospital Dispensary. *d*-Benzphetamine was synthesized and kindly provided by Dr Björn Lindeke, Department of Organic Pharmacy, University of Uppsala, from norbenzphetamine by the Eschweiler Clarke reaction, and, as judged by IR, NMR and mass spectroscopy analysis, in accordance with known properties of this substance. All other chemicals were of analytical grade and obtained from local commercial sources.

Drosophila *strains*. The wild type strains Berlin-K, Canton S, Florida-9 (aflatoxin B_1-sensitive) and Karsnäs 60_w (an isogenic strain synthesized from the original Swedish Karsnäs 60 by brother–sister crosses, carrying the markers $w_{(x)}$ and $B^S_{(y)}$, provided by Professor K. N. Lüning, Stockholm) were used in this study. In addition, Oregon R (R) (resistant to insecticides through DDT selection since 1952, provided by Dr D. J. Merrel, Minnesota), Hikone R (cross-resistant to several classes of insecticides, provided by Dr E. Vogel, Leiden, The Netherlands), Lausanne-S (aflatoxin B_1-resistant, provided by Dr

Table 1. Cytochrome P-450-dependent oxidative demethylation by microsomes from different *Drosophila melanogaster* strains

Strain	nmoles formaldehyde formed/mg microsomal protein per min*		
	Aminopyrine	Ethylmorphine	Benzphetamine
Karsnäs 60_w	1.5 ± 0.3†	1.0 ± 0.2	1.2 ± 0.2
Hikone R§	2.1 ± 0.2	0.6 ± 0.1	0.6 ± 0.1
Florida-9‖	2.4 ± 0.6	2.3 ± 0.6	2.7 ± 0.3
Lausanne S¶	1.4 ± 0.3	1.0 ± 0.2	1.4 ± 0.3
Canton S	1.1 ± 0.3	0.8 ± 0.2	1.3 ± 0.3
Eth-29**	1.5 ± 0.3	1.4 ± 0.4	2.0 ± 0.4
Berlin K	0.9 ± 0.1	0.7 ± 0.2	ND‡
Oregon R§	3.8 ± 0.6	1.4 ± 0.3	1.8 ± 0.4
Marked inversion	1.0 ± 0.1	0.6 ± 0.1	ND

* Endogenous formaldehyde production, determined from incubated samples containing microsomes and cofactors but no substrate, is subtracted from each data. The production is dependent of protein content, is about 0.5 nmol/mg protein per min and shows no particular strain variation.

† The data represent mean ± SE of at least three experiments.

‡ Not determined.

§ Insecticide resistant.

‖ AFB_1-sensitive.

¶ AFB_1-resistant.

** Halothane and ether resistant.

J. Chinnici, U.S.A.) and Eth-29 (resistant to ether, halothane and chloroform, provided by Dr S. Gano, Japan) was used. For the genetic analysis a strain was used called Marked Inversion (M, In(1)$sc^{8L}y^{3PR}$ + S, $y^{S1}y^{3P}sc^{8}$: In (2L + R)Cy, al^{2}Cy lt^{3} sp^{2}/In(2LR)Pm, dp b Pm^{1}: In(3LR)DCxF, ru h D ca/In(3R) Sb, obtained from the Institute of Genetics, University of Stockholm, Sweden) and appropriate hybrids between Oregon R and Marked Inversion, carrying homoor hemizygous Oregon R X chromosome and in heterozygous form the second, second + third, third or no Oregon R autosomes (see Table 3).

Preparation of microsomes. Two-to-five-day-old flies were homogenized in ice-cold 0.1 M sodium phosphate buffer pH 7.5 containing 1 mM EDTA in a Glass-Teflon Potter–Elvehjem homogenizer (0.1–0.2 g flies/ml). The homogenate was centrifuged for 10 min at 15,000 *g* and the supernatant filtered through nylon cloth, layered over 0.3 M sucrose in the same buffer (according to Arrhenius [17]) and centrifuged at 115,000 *g* for 60 min. The microsomal pellet was resuspended in buffer (about 10 mg microsomal protein, corresponding to about 2000 flies per ml). The temperature was kept at about 2° during the whole preparation. The microsomes were used for enzymatic assays within 1 hr, or rapidly frozen at −70°, which caused negligible loss of enzyme activity during storage up to 2–3 months.

Assays. The demethylation reactions were recorded by means of formaldehyde production estimated by the Hantzsch reaction [18]. The incubation mixture contained 0.25 μmol $NADP^+$, 5 μmol glucose-6-phosphate, 0.5 units glucose-6-phosphate dehydrogenase, 30 μmol nicotine amide, 1–4 mg microsomal protein and 5 μmol aminopyrine or ethylmorphine or 200 μmol benzphetamine in 1 ml. The samples were prewarmed for 2 min and incubated for 10 min at 37° after the addition of substrate, and the reaction was stopped by the addition of 0.35 ml 15% TCA. 1 ml Nash reagent was added to 1 ml of the centrifuged sample, warmed at 60° for 10 min and the formaldehyde production was recorded at 412 nm. The microsomes formed significant amounts of formaldehyde in the absence of substrate (see footnote, Table 1); the absorbance of incubated samples without substrate has been subtracted from each value. The protein content of the microsomes was determined according to Lowry [19].

RESULTS AND DISCUSSION

The basal capacity to *N*-demethylate the three substrates varied by a factor of four to five between the different strains studied (Table 1). For aminopyrine *N*-demethylase activity the highest value was noted for the insecticide-resistant strain Oregon R, with high metabolism also in Florida-9 and Hikone R. Microsomes from Berlin K had the lowest capacity for this reaction. The metabolism of both ethylmorphine and *d*-benzphetamine was highest in the AFB_1-sensitive Florida-9. It is likely that this high *N*-demethylation capacity when compared with the AFB_1-tolerant Lausanne S is of importance for the AFB_1-sensitivity [20] as AFB_1 is activated via the cytochrome P-450 system to toxic and carcinogenic metabolites [21, 22]; this is the only metabolic difference observed so far between Florida-9 and Lausanne S. A low *N*-demethylase activity is observed in Hikone R, Berlin K and the marker strain with ethylmorphine as substrate, and in Hikone R also with *d*-benzphetamine (Berlin K and the marker strain is, however, untested for this reaction). In Table 2 the genetic variation in the above mentioned *N*-demethylations is compared, by means of the correlation coefficients, with each other and with several previously studied metabolic activities. The capacity to *N*-demethylate *d*-benzphetamine and ethylmorphine is closely correlated in the different strains ($P < 0.001$), indicating that they are either mainly metabolized by the same cytochrome P-450 form, or

Table 2. Correlation coefficient for different cytochrome P-450-dependent activities in *Drosophila melanogaster**

	Aminopyrine demethylation	Benzphetamine demethylation	Ethylmorphine demethylation
Aminopyrine demethylation	—	0.29	0.53
Benzphetamine demethylation	0.29	—	0.96†
Ethylmorphine demethylation	0.53	0.96†	—
p-Nitroanisole demethylation	0.70	0.26	0.18
Biphenyl 3-hydroxylation	0.67	0.24	0.01
Biphenyl 4-hydroxylation	0.93†	0.08	0.17
Benzo(*a*)pyrene hydroxylation	0.31	0.47	0.13
7-Ethoxycoumarin deethylation	0.06	0.54	0.23

* The estimation of the correlation coefficients is based on data on the enzymatic activities in 7 to 9, as far as can be judged unrelated, *Drosophila* strains. The data is from the present paper and from reference [12].

† $P < 0.001$.

Table 3. Aminopyrine demethylation in hybrids between Oregon R and the marker strain M (y^3p/Y Cy/Pm D/Sb)

Genotype	Chromosomes from Oregon R	Aminopyrine demethylase activity, nmol formaldehyde formed/mg microsomal protein per min
OR/Cy D/Sb[a]	2	2.5 ± 0.5
OR/Cy OR/D	2.3	2.1 ± 0.4
Cy/Pm OR/D	3	1.0 ± 0.2
Cy/Pm D/Sb	—	1.2 ± 0.3
Oregon R	2.3	3.8 ± 0.6
M (Cy/Pm D/Sb)	—	1.0 ± 0.1

* The flies also carry the X chromosome from the Oregon strain.

by different forms under the same regulatory control. They are not correlated to any other activity studied so far, and may represent a separate cytochrome P-450 form or a class of cytochrome P-450 enzymes.

A metabolic resistance to insecticides in *Drosophila* as well as the house fly, *Musca domestica*, is shown to give rise to cross-resistance to several classes of insecticides and increased capacity for specific P-450-dependent reactions [10, 12, 14, 15, 23–27] in uninduced flies. The resistance is suggested to be due to a change in a regulatory gene in both organisms [14, 15, 28, 29] probably producing an insecticide-binding receptor [28] in analogy with the Ah-receptor in mice [30, 31] regulating several cytochrome P-450-dependent reactions. The three *N*-demethylation reactions in this study seem not to be associated with this insecticide resistance gene (the RI gene) in *Drosophila*, although several other demethylation reactions of i.e. *p*-nitroanisole [10, 12, 14, 15] and dimethylnitrosamine [13, 16] are associated.

Aminopyrine *N*-demethylation is correlated with the hydroxylation of biphenyl in 4-position ($P < 0.001$). A study of the genetics of aminopyrine metabolism (Table 3) revealed the high metabolism in Oregon R to be apparently semidominantly inherited by means of the intermediate activity in the heterozygous hybrids. The higher activity in the hybrids carrying a second chromosome from the Oregon R parent strain demonstrated that the high activity was regulated by the second chromosome, in accordance with previous results concerning biphenyl 4-hydroxylation [14, 15]. These similarities support the idea that the Oregon R strain might have a change in a regulatory gene, giving rise to an increased biphenyl 4-hydroxylase, and apparently also aminopyrine *N*-demethylase activity. The regulator would be acting in *trans* as it is localized to a mid-position on the second chromosome, and a possible structural gene determining low biphenyl 4-hydroxylase activity in Berlin K is located to a proximal position in the same chromosome [15]. Further studies are needed to characterize the regulatory gene, and to localize possible structural genes determining aminopyrine *N*-demethylation capacity.

The low *N*-demethylase activity in Berlin K with aminopyrine and ethylmorphine as substrate and in Hikone R with ethylmorphine and *d*-benzphetamine has significance for their use as the probably most common test strains in mutagenicity testing. They have both been shown to have a markedly lower metabolism of several substrates than other strains and should maybe be regarded as less fitted for the detection of indirect mutagens. Florida-9, Karsnäs 60 and especially Oregon R (R) appear to have mean capacities or higher for the metabolic reactions studied so far, and could represent a better choice when the mutagenic effects of a compound with unknown metabolism is analysed.

In conclusion, the present study has revealed a marked variation in the capacity to perform three cytochrome P-450-dependent demethylations.

Genetically-determined variation in the cytochrome P-450 enzymes might represent an important feature for the adaptation to a changing environment with the widespread contamination with xenobiotics present today. The genetic correlation between *d*-benzphetamine and ethylmorphine metabolism as well as the apparently common, second chromosome-dependent regulation of biphenyl 4-hydroxylase activity and aminopyrine *N*-demethylation provide a basis for further genetic analyses, contributing to the understanding of the genetics of the cytochrome P-450 system in *Drosophila*.

Acknowledgements—The author wishes to thank Professor C. Ramel for stimulating discussion. This work has been supported by grants from the Swedish Council for Planning and Coordination of Research and the Swedish National Environmental Protection Board.

REFERENCES

1. E. Vogel and F. H. Sobels, in *Chemical Mutagens, Principles and Methods for their Detection* 4 (Ed. A. Hollaender), p. 93. Plenum Press, New York (1976).
2. F. E. Würgler, F. H. Sobels and E. Vogel, in *Handbook of Mutagenicity Test Procedures* (Eds. B. J. Kilbey, M. Legator, W. Nichols and C. Ramel), p. 335. Elsevier/North Holland Biomedical Press, Amsterdam (1977).
3. E. Vogel, W. G. H. Blijleven, P. M. Klapwijk and J. A. Ziljstra, in *The Predictive Value of Short-Term Screening Tests in Carcinogenicity Evaluation* (Ed. G. M. Williams), p. 125. Elsevier/North Holland Biomedical Press, Amsterdam (1980).
4. A. J. Baars, J. A. Ziljstra, E. Vogel and D. D. Breimer, *Mutat. Res.* **44**, 257 (1977).
5. A. J. Baars, *Drug Metab. Rev.* **11**, 191 (1980).
6. J. Hällström and R. Grafström, *Chem.-biol. Interact.* **34**, 145 (1981).
7. I. Hällström, A. Blanck and S. Atuma, *Chem.-biol. Interact.* **46**, 39 (1983).
8. C. Naquira, R. A. White and M. Agoson, in *Biochemistry, Biophysics and Regulation of Cytochrome P-450* (Eds. J.-Å. Gustafsson, J. Carlstedt-Duke, A. Mode and J. Rafter), p. 105. Elsevier/North Holland Biomedical Press, Amsterdam (1980).
9. I. Hällström, *Mutat. Res.* **174**, 93 (1986).
10. J.-A. Ziljstra, E. Vogel and D. D. Breimer, *Chem.-Biol. Interact.* **48**, 317 (1984).
11. S. W. Bigelow, J. A. Ziljstra, E. Vogel and D. W. Nebert, *Archs Toxic.* **56**, 219 (1985).
12. I. Hällström, A. Blanck and S. Atuma, *Biochem. Pharmac.* **33**, 13 (1984).
13. L. C. Waters, C. E. Nix and J. L. Epler, *Chem.-Biol. Interact.* **46**, 55 (1983).
14. I. Hällström and A. Blanck, *Chem.-Biol. Interact.* **56**, 157 (1985).
15. I. Hällström, *Chem.-Biol. Interact.* **56**, 173 (1985).
16. I. Hällström, J. Magnusson and C. Ramel, *Mutat. Res.* **92**, 161 (1982).
17. E. Arrhenius, *Cancer Res.* **28**, 264 (1968).
18. T. Nash, *Biochemistry* **55**, 416 (1953).
19. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
20. J. P. Chinnici, *J. Hered.* **71**, 275 (1980).
21. E. K. Weisburger, *Mol. Cell Biochem.* **32**, 95 (1980).
22. E. C. Miller and J. A. Miller, *Cancer* **47**, 2327 (1981).
23. H. Kikkawa, *Ann. Rep. Sci. Works Fac. Sci., Osaka Univ.* **9**, 1 (1961).
24. L. G. Tate, F. W. Plapp and E. Hodgson, *Chem.-Biol. Interact.* **6**, 237 (1973).
25. M. Agosin, *Mol. Cell Biochem.* **12**, 33 (1976).
26. E. Hodgson, L. G. Tate, A. P. Kulkarni and F. W. Plapp, *Agr. Food Chem.* **22**, 360 (1974).
27. S. J. Yu and L. C. Terriere, *Pesticide Biochem. Physiol.* **12**, 239 (1979).
28. F. W. Plapp, *Pesticide Biochem. Physiol.* **22**, 194 (1984).
29. F. W. Plapp and J. S. Johnston, in *The Evolutionary Significance of Insect Polymorphism* (Eds. M. W. Stock and A. C. Bartlett), Forest, Wildl. and Range Exp. Sta., Univ. Idaho, Moscow (1982).
30. D. W. Nebert and J. S. Felton, *Fedn Proc. Fedn Am. Socs. exp. Biol.* **35**, 1133 (1976).
31. A. Poland and E. Glover, *J. biol. Chem.* **251**, 4936 (1976).