WB-4101 does indeed interact in a concentration-dependent fashion with the dihydropyridine receptor in myocardial tissue. This interaction occurred with specifically rather than non-specifically bound [$^3$H]nitrendipine as suggested by the stereoselectivity of WB-4101 and the experiments demonstrating that racemic WB-4101 did not alter non-specifically bound [$^3$H]nitrendipine. Concentrations of WB-4101 ranging between $10^{-5}$ and $10^{-4}$ M are approximately 100-fold higher than needed to evoke $\alpha_1$-adrenergic blocking effects [5, 9, 11]. However, the concentrations are similar to those observed by Atlas and Adler [5] to displace [$^3$H]WB-4101 from its binding sites in neuroblastoma glioma cells, suggesting that the low affinity binding site of [$^3$H]WB-4101 in these cells may represent the dihydropyridine receptor.

Because of the high concentrations needed and the limited amounts of compounds, the data allow us to conclude that specific rather than non-specific binding is being altered, but do not allow us to infer whether the mechanism is competitive or allosteric. Nevertheless, the interaction of WB-4101 with the [$^3$H]nitrendipine binding site may be related to the calcium channel blocking effects of this compound as is suggested by the similar pharmacologic and binding $IC_{50}$ values ($0.9 \times 10^{-4}$ M compared to $0.73 \times 10^{-4}$ M). However, other mechanisms for the pharmacologic response of WB-4101 in these experiments cannot be excluded.

In conclusion, our results demonstrate that WB-4101, a potent $\alpha_1$-adrenergic blocking agent, also interacted specifically with the dihydropyridine calcium channel receptor. The potency of WB-4101 at the dihydropyridine receptor correlated with its pharmacologic potency as a calcium channel blocker. Because of the high concentrations required for calcium channel blocking activity, the implications of these findings to studies performed at concentrations required to elicit $\alpha_1$-adrenergic blocking effects are not important. However, the observations may be important for understanding the toxicities of WB-4101 and perhaps other $\alpha$-adrenergic blocking agents. In addition, the study has important implications for pharmacologic studies employing adrenergic blocking agents. In particular, $\alpha_1$-adrenergic blocking agents such as WB-4101 may be used in pharmacologic studies to "block" adrenergic receptors. In these situations, it is important to understand that at high concentrations $\alpha_1$-adrenergic blocking agents may also inhibit calcium channels. Thus, the interpretation of the pharmacologic specificity of $\alpha_1$-adrenergic blocking agents at higher concentrations should be made with the knowledge that these concentrations may also invoke calcium channel blocking effects.

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REFERENCES

1. A. S. Fairhurst, M. L. Whittaker and F. J. Ehlert, *Biochem. Pharmac.* **29**, 155 (1980).
2. J. S. Karliner, H. J. Motulsky, J. Dunlap, J. H. Brown and P. A. Insel, *J. cardiovasc. Pharmac.* **4**, 515 (1982).
3. W. G. Nayler, J. E. Thompson and B. Jarrott, *J. molec. cell. Cardiol.* **14**, 185 (1982).
4. H. J. Motulsky, M. D. Snavely, R. J. Hughes and P. A. Insel, *Circulation Res.* **52**, 226 (1983).
5. D. Atlas and M. Adler, *Proc. natn. Acad. Sci. U.S.A.* **78**, 1237 (1981).
6. K. M. M. Murphy and S. H. Snyder, *Eur. J. Pharmac.* **77**, 201 (1982).
7. L. T. Williams and P. Tremble, *J. clin. Invest.* **70**, 209 (1982).
8. G. T. Bolger, P. Gengo, R. Klockowski, E. Luchowski, H. Siegel, R. A. Janis, A. M. Triggle and D. J. Triggle, *J. Pharmac. exp. Ther.* **225**, 291 (1983).
9. W. L. Nelson, J. E. Wennerstrom, D. C. Dyer and M. Engel, *J. med. Chem.* **20**, 880 (1977).
10. W. L. Nelson, M. L. Powell and D. C. Dyer, *J. med. Chem.* **22**, 1125 (1979).
11. R. R. Ruffolo, W. L. Nelson and E. L. Yaden, *Naunyn-Schmiedeberg's Archs Pharmac.* **322**, 93 (1983).

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# An example of P-450 catalytic activities not correlated with corresponding P-450 mRNA concentrations

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Enzyme induction is usually associated with elevated levels of messenger RNA [reviewed in Refs. 1 and 2]. Increased mRNA concentrations can be the result of *de novo* synthesis (transcriptional activation of the gene), enhanced mRNA stabilization, or some combination of both. Likewise, increased catalytic activity can be the result of *de novo* protein synthesis, enhanced stabilization of the active protein, or some combination of both.

The induction of P-450 by phenobarbital [3–6] and polycyclic aromatic compounds such as 3-methylcholanthrene or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin [7–10] has been shown to be principally due to transcriptional activation of the corresponding genes. At maximally induced $P_1$-450 mRNA levels in mouse liver, inducible aryl hydrocarbon hydroxylase activity appears to reach a plateau, however, suggesting that translational processes may be involved in limiting the expression of the enzyme activity [11]. To our knowledge this study is the only reported example [11] in which P-450 mRNA levels have been shown not always to be correlated with their corresponding catalytic activity. The purpose of this report is to illustrate another example.

In this study, high intraperitoneal doses of isosafrole were given to the C57BL/6N mouse. Hepatic $P_1$-450 and $P_3$-450 mRNA concentrations were elevated markedly, while their corresponding catalytic activities ($P_1$-450 = aryl

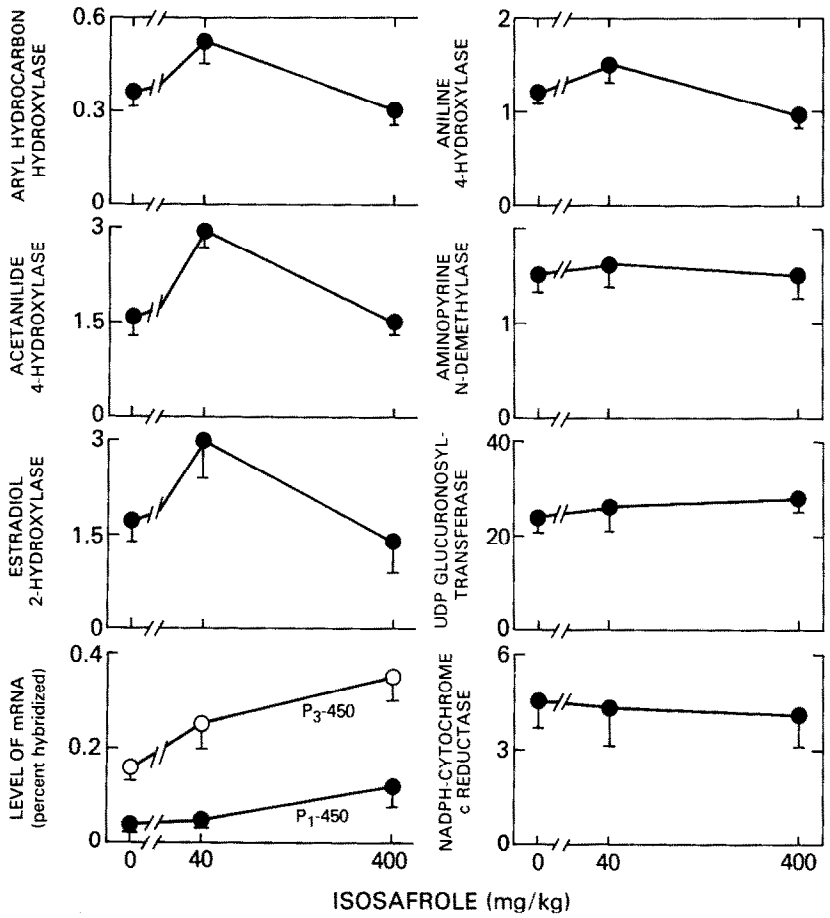


Fig. 1. Five P-450-mediated monooxygenase activities, one UDP glucuronosyltransferase activity, NADPH-cytochrome *c* reductase activity, and $P_1$-450 and $P_3$-450 mRNA levels in C57BL/6N liver as a function of isosafrole dose given 24 hr before killing. *Symbols* and *brackets* denote means ± S.D. Each value was determined in duplicate on three different occasions. Enzyme activities were determined in duplicate on three different groups (N = 4) of animals. Aryl hydrocarbon hydroxylase [16], acetanilide 4-hydroxylase [17], estradiol 2-hydroxylase [18], aniline 4-hydroxylase [19], aminopyrine *N*-demethylase [20, 21], UDP glucuronosyltransferase with 4-methylumbelliferone as substrate [22], and NADPH-cytochrome *c* reductase [23] assays were carried out by the methods cited. One unit of activity represents 1 nmole of product formed in 1 min at 37°. Specific activity denotes units per mg microsomal protein, except in the case of the reductase in which specific activity denotes units of reduced cytochrome *c* per nmole P-450 at 25°. Microsomal P-450 concentration was measured by the method of Omura and Sato [24]. Poly(A)-enriched RNA was prepared by means of guanidine–HCl extraction [25] and one pass over oligo(dT)-cellulose chromatography [26] with an intermediate heat step [27]. $P_1$-450 and $P_3$-450 cDNA inserts (1259 and 1304 base pairs respectively), each known to be 3′-specific for their corresponding mRNA following sequence analysis [28], were electroeluted and fixed to nitrocellulose filters by the methods described [29, 30]. Poly(A)-enriched RNA was then partially digested with 0.2 N NaOH, end-labeled with [$\gamma$-$^{32}$P]UTP, and hybridized to nitrocellulose filters under the conditions described [30]. Controls (background) represented radioactivity eluted from pBR322 DNA-bound filters. The percent of total mRNA hybridized (experimental minus control) was then determined under conditions previously described [3, 8, 30].

hydrocarbon hydroxylase; $P_3$-450 = acetanilide 4-hydroxylase and estradiol* 2-hydroxylase) were not. Other membrane-bound enzymes (aniline 4-hydroxylase, aminopyrine *N*-demethylase, UDP glucuronosyltransferase, and NADPH-cytochrome *c* reductase) were not affected. The data provide additional evidence that elevated P-450 mRNA levels need not be correlated with their corresponding P-450 activities, despite no evidence of hepatic toxicity or microsomal membrane damage.

*Treatment of mice and preparation of microsomes.* Sexually immature (4- to 5-weeks-old) C57BL/6N mice of either sex were used. Isosafrole (40 and 400 mg/kg) in corn oil (25 ml/kg) was administered as a single intraperitoneal dose 24 hr before killing. It was first established that these doses of isosafrole induced maximal levels of $P_1$-450 and $P_3$-450 mRNA between 6 and 24 hr of drug treatment. Controls received corn oil alone. Liver microsomes were prepared as described previously [12] and stored in 0.5-ml aliquots at −80° until assayed. Protein concentrations were determined by the method of Lowry *et al.* [13] with bovine serum albumin as the standard.

*Lack of correlation between catalytic activity and corresponding messenger.* Aryl hydrocarbon hydroxylase activity is best correlated with mouse $P_1$-450, whereas acetanilide 4-hydroxylase and estradiol 2-hydroxylase activities

* Abbreviation used: estradiol, 1,3,5(10)-estratriene-3,17$\beta$-diol.

are best correlated with mouse $P_3$-450 [14, 15]. Striking correlations are generally found between the catalytic activity and corresponding mRNA during studies involving developmental and tissue specificity of the expression of these two genes [15].

Figure 1 shows that these correlations between either $P_1$-450 or $P_3$-450 mRNA and their corresponding activities do not exist following treatment with large doses of the P-450 inducer isosafrole. Because these three activities depend on a membrane-bound multicomponent electron chain [31], one possible explanation is that isosafrole at high doses causes damage to the endoplasmic reticulum such that these activities are affected. This hypothesis was thus explored (Fig. 1) with two additional microsomal P-450-mediated activities (aniline 4-hydroxylase and aminopyrine *N*-demethylase) and two other membrane-bound activities (NADPH-cytochrome *c* reductase and UDP glucuronosyltransferase). In each instance the activity at the highest isosafrole dosage was lowered but not significantly less than the basal activity, providing no definitive evidence for severe cellular toxicity or membrane damage. The liver appeared morphologically normal, and the tissue was normal by light microscopic examination.

*Conclusion.* The data in this report thus illustrate a situation in which the quantities of cytosolic (and presumably translatable) $P_1$-450 and $P_3$-450 mRNA are not correlated with the levels of their corresponding catalytic activities. The mechanism for this observation may be related to the fact that isosafrole induces, and in turn is metabolized by, $P_1$-450 and $P_3$-450 more so than the forms of P-450 responsible for aniline hydroxylase or aminopyrine *N*-demethylase [32, 33]. In turn, formation of stable metabolite–inhibitor complexes with specific forms of P-450 [34, 35] could account for decreased $P_1$-450 and $P_3$-450 catalytic activity in the face of large amounts of mRNA. This hypothesis would suggest that ample amounts of inactive $P_1$-450 and $P_3$-450 protein are being translated from mRNA and incorporated into the endoplasmic reticulum. Since $P_3$-450 metabolizes isosafrole considerably more than $P_1$-450 [33], one would expect that $P_3$-450 would be far more damaged than $P_1$-450; however, this was not found in terms of the corresponding catalytic activities (Fig. 1). It would be of interest to study these mice with monoclonal antibodies specific for mouse $P_1$-450 and for mouse $P_3$-450 proteins.

It is reasonable to predict that there will be more examples reported in which P-450 mRNA concentrations do not reflect the true level of catalytically active P-450 protein. This possibility should be looked for and guarded against, especially in the field of pharmacology or toxicology where large doses of potentially toxic drugs and other chemicals are given to the laboratory animal.

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REFERENCES

1. A. L. Goldberg and A. C. St. John, *A. Rev. Biochem.* **45**, 747 (1976).
2. T. Omura, *Pharmac. Ther.* **8**, 489 (1980).
3. F. J. Gonzalez and C. B. Kasper, *J. biol. Chem.* **257**, 5962 (1982).
4. Y. Fujii-Kuriyama, Y. Mizukami, K. Kawajiri, K. Sogawa and M. Muramatsu, *Proc. natn. Acad. Sci. U.S.A.* **79**, 2793 (1982).
5. J. P. Hardwick, F. J. Gonzalez and C. B. Kasper, *J. biol. Chem.* **258**, 8081 (1983).
6. M. Adesnik, S. Bar-Nun, F. Maschio, M. Zunich, A. Lippman and E. Bard, *J. biol. Chem.* **256**, 10340 (1981).
7. R. H. Tukey, D. W. Nebert and M. Negishi, *J. biol. Chem.* **256**, 6969 (1981).
8. F. J. Gonzalez, R. H. Tukey and D. W. Nebert, *Molec. Pharmac.* **26**, 117 (1984).
9. D. I. Israel and J. P. Whitlock, Jr., *J. biol. Chem.* **259**, 5400 (1984).
10. K. Kawajiri, O. Gotoh, Y. Tagashira, K. Sogawa and Y. Fujii-Kuriyama, *J. biol. Chem.* **259**, 10145 (1984).
11. R. H. Tukey, M. Negishi and D. W. Nebert, *Molec. Pharmac.* **22**, 779 (1982).
12. S. A. Atlas, A. R. Boobis, J. S. Felton, S. S. Thorgeirsson and D. W. Nebert, *J. biol. Chem.* **252**, 4712 (1977).
13. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
14. D. W. Nebert, S. Kimura and F. J. Gonzalez, *Molecular Biology of Development* (Eds. E. H. Davidson and R. A. Firtel), p. 309. Alan R. Liss, New York (1984).
15. N. Tuteja, F. J. Gonzalez and D. W. Nebert, *Devl Biol.* **112**, 177 (1984).
16. D. W. Nebert, *Meth. Enzym.* **LII**, 226 (1978).
17. T. M. Guenthner, M. Negishi and D. W. Nebert, *Analyt. Biochem.* **96**, 201 (1979).
18. M. Numazawa, Y. Kiyono and T. Nambara, *Analyt. Biochem.* **104**, 290 (1980).
19. Y. Imai and R. Sato, *J. Biochem., Tokyo* **60**, 417 (1966).
20. T. Nash, *Biochem. J.* **55**, 416 (1953).
21. A. P. Poland and D. W. Nebert, *J. Pharmac. exp. Ther.* **184**, 227 (1973).
22. P. I. Mackenzie and I. S. Owens, *Biochem. Pharmac.* **32**, 3777 (1983).
23. J. J. Zimmerman, J. P. Gorski and C. B. Kasper, *Drug Metab. Dispos.* **5**, 572 (1977).
24. T. Omura and R. Sato, *J. biol. Chem.* **239**, 2370 (1964).
25. R. A. Cox, *Meth. Enzym.* **12**, 120 (1968).
26. H. Aviv and P. Leder, *Proc. natn. Acad. Sci. U.S.A.* **69**, 1408 (1972).
27. F. J. Gonzalez and C. B. Kasper, *Biochemistry* **20**, 2292 (1981).
28. S. Kimura, F. J. Gonzalez and D. W. Nebert, *J. biol. Chem.* **259**, 10705 (1984).
29. M. Melli, E. Ginelli, G. Corneo and R. Dilernia, *J. molec. Biol.* **93**, 23 (1975).
30. A. C. Spradling, M. E. Digan, A. P. Mahowald, M. Scott and E. A. Craig, *Cell* **19**, 905 (1980).
31. G. J. Mannering, in *Concepts in Drug Metabolism* (Eds. P. Jenner and B. Testa), p. 53. Marcel-Dekker, New York (1981).
32. M. Negishi and D. W. Nebert, *J. biol. Chem.* **254**, 11015 (1979).
33. T. Ohyama, D. W. Nebert and M. Negishi, *J. biol. Chem.* **259**, 2675 (1984).
34. M. R. Franklin, *Xenobiotica* **1**, 581 (1971).
35. C. R. Elcombe, J. W. Bridges, T. J. B. Gray, R. H. Nimmo-Smith and K. J. Netter, *Biochem. Pharmac.* **24**, 1427 (1975).