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Differential Inducibility of Nuclear Envelope Epoxide Hydratase by *Trans*-stilbene Oxide and Phenobarbital

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SUMMARY

Rough and smooth microsomal membranes and nuclear envelope were isolated from rat liver following the administration of either *trans*-stilbene oxide or phenobarbital. These membranes were assayed for epoxide hydratase activity using styrene oxide and benzo[*a*]pyrene 4,5-oxide as substrates. Activity toward both substrates was increased in rough and smooth endoplasmic reticulum approximately 3.0- to 3.5-fold above control values in *trans*-stilbene oxide-treated animals. Phenobarbital also increased levels of both epoxide hydratase activities in these two membrane systems, but to a lesser extent (2.0- to 2.5-fold). In contrast to the results obtained with endoplasmic reticulum, only *trans*-stilbene oxide administration elevated levels of epoxide hydratase activity in nuclear envelope; activity toward styrene oxide and benzo[*a*]pyrene 4,5-oxide increased 3.0- and 2.4-fold above control levels, respectively. Administration of phenobarbital, however, slightly decreased nuclear envelope activity toward styrene oxide but did not significantly alter activity toward benzo[*a*]pyrene 4,5-oxide. Quantitative immunochemical studies further demonstrated that nuclear envelope epoxide hydratase is unresponsive to induction by phenobarbital. This conclusion is also supported by gel electrophoretic analysis of nuclear envelope from control and induced animals. In addition, microsomal UDP-glucuronosyltransferase, NADPH-cytochrome *c* oxidoreductase, and cytochrome P-450 were increased by both inducing agents, whereas their nuclear envelope counterparts remained refractive to phenobarbital induction. Hence, even though *trans*-stilbene oxide and phenobarbital induce similar spectra of enzymes and cytochromes in the endoplasmic reticulum, only *trans*-stilbene oxide induces nuclear envelope epoxide hydratase. These observations lend further support to the position that the cellular controls regulating levels of nuclear envelope enzymes differ from those operative for the endoplasmic reticulum and direct attention to intriguing differences between these agents in their mechanisms of action.

INTRODUCTION

The nuclear envelope is composed of an inner and an outer membrane, each containing the characteristic phospholipid bilayer common to other intracellular membranes. An interesting structural feature of the envelope is the pore complex, which is thought to represent the major avenue for nucleocytoplasmic transport. In addition, the outer leaflet of the nuclear envelope is continuous with the endoplasmic reticulum, and both membrane systems share many of the same enzymes. Among these are UDP-glucuronosyltransferase, NADH-cytochrome b_5 oxidoreductase, cytochrome b_5, Mg^{2+}-ATPase, glucose 6-phosphatase, cytochrome P-450, cytochrome P-448, NADPH-cytochrome *c* oxidoreductase, and epoxide hydratase (1–3). The latter three nuclear envelope enzymes have each been shown to have all of the immunological determinants of their endoplasmic reticulum counterparts (4, 5), suggesting that those enzymatic activities shared by both membrane systems are associated with the same molecular entities. Other ultrastructural, physicochemical, and biochemical characteristics of the nuclear envelope have been discussed (1, 3, 6, 7).

Phenobarbital and 3-methylcholanthrene are representative of two classes of inducing agents that increase levels of certain microsomal enzymes and cytochromes (8). The latter class, which also includes such compounds as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, associates with a unique receptor (9) that interacts with nuclear components to increase levels of certain mRNAs (10–12). A single injection of phenobarbital was recently shown to increase levels of mRNAs coding for the inducible enzymes NADPH-cytochrome *c* oxidoreductase (13) and epoxide hydratase (14). Others (15) found that levels of epoxide hydratase mRNA remain elevated after chronic administration of the drug. The increase in these mRNAs appears to be the result of the stimulation by phenobar-

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bital of transcriptional and/or post-transcriptional nuclear events (13, 14). However, no receptor for phenobarbital has been described.

Although each of the two classes of inducing agents acts by increasing levels of its own unique spectrum of mRNAs, several differences have been noted in the physiological response to these agents. One such difference is the proliferation of smooth endoplasmic reticulum and general liver hypertrophy, which occurs upon chronic administration of phenobarbital but is absent in the 3-methylcholanthrene response (8, 16, 17). Another important biochemical difference is the lack of inducibility of certain nuclear envelope enzymes whose counterparts are induced in the endoplasmic reticulum by phenobarbital (2, 18–21). 3-Methylcholanthrene, however, increases levels of aryl hydrocarbon hydroxylase activity and cytochrome P-448 associated with both membrane systems (2, 19, 21). In view of this latter difference, other inducing agents were sought that could be used to explore the molecular mechanism underlying the lack of inducibility of certain nuclear envelope enzymes by phenobarbital. Recently, *trans*-stilbene oxide was found to induce a spectrum of enzymes similar to that of phenobarbital (22, 23) and was further shown to increase levels of nuclear epoxide hydratase (24). This report describes a detailed analysis of the effects of *trans*-stilbene oxide and phenobarbital administration on the levels of specific nuclear envelope enzymes and compares these responses with those noted for the rough and smooth endoplasmic reticulum.

MATERIALS AND METHODS

Animals. Young adult male Sprague-Dawley rats (150–170 g) were given i.p. injections of *trans*-stilbene oxide (600 mg/kg), dissolved in corn oil 24 hr prior to being killed. Control animals received corn oil alone. Phenobarbital (10 mg/kg) was injected i.p. for 4 consecutive days at 24-hr intervals, and the rats were killed 18 hr after the final injection. In this case the control animals received 0.15 M NaCl. All animals were deprived of food for 18 hr prior to death.

Membrane purification. Nuclear envelope was purified according to the method of Kasper (25), and rough and smooth microsomes were isolated as described by Dallner (26), with modifications. The lower sucrose cushion was 1.38 M sucrose containing 15 mM $CsCl_2$, and the gradients were centrifuged at 100,000 × *g* for 20 hr in a Beckman SW 25.2 rotor at 4°. Each fraction of endoplasmic reticulum was washed with 10% potassium citrate followed by twice-distilled water as previously described (27).

Enzyme assays. Epoxide hydratase assays using benzo[*a*]pyrene 4,5-oxide as a substrate were performed as described by Jerina *et al.* (28). The epoxide hydratase assay outlined by Lyman *et al.* (29) was used when the substrate was styrene oxide. UDP-glucuronosyltransferase and NADPH-cytochrome *c* oxidoreductase assays were performed as described previously (30), except that digitonin (0.2%) was added to the transferase assays for maximal activation (31). Cytochromes P-450 and b_5 were quantitated as previously described (18).

Immunochemical measurement of epoxide hydratase. Epoxide hydratase was quantitated by radial immunodiffusion (4) with purified epoxide hydratase (14) as standard. Membranes were solubilized with a solution containing 1% Triton X-100 and 1% deoxycholate, and 1% NP-40 (BDH Chemicals) was incorporated into the agar used for immunodiffusion.

RESULTS

Effects of trans-stilbene oxide and phenobarbital on epoxide hydratase, NADPH-cytochrome c oxidoreductase, UDP-glucuronosyltransferase, cytochrome b_5, and cytochrome P-450 in rough and smooth microsomal membranes. Tables 1 and 2 reveal that both phenobarbital and *trans*-stilbene increase the enzymatic specific activities of epoxide hydratase in rough and smooth microsomes. This increase is evident when either styrene oxide or benzo[*a*]pyrene 4,5-oxide is used as a substrate; however, *trans*-stilbene oxide induces epoxide hydratase activity slightly more than does phenobarbital. In addition, levels of total cytochrome P-450 were increased by both inducing agents, with phenobarbital producing a 3-fold increase in the smooth microsomes, compared with an approximate 2-fold increase in rough microsomes. In

TABLE 1

Effects of phenobarbital on epoxide hydratase, NADPH-cytochrome c oxidoreductase, UDP-glucuronosyltransferase, cytochrome b_5, and cytochrome P-450 contents in rough and smooth microsomal membrane[a]

Enzyme	Specific activity[b]					
	Rough microsomal membrane			Smooth microsomal membrane		
	Control	Induced	Induced/control	Control	Induced	Induced/control
Epoxide hydratase						
Styrene oxide	28.3 ± 3.0	60.4 ± 1.3	2.1	33.1 ± 0.9	86.7 ± 8.3	2.6
Benzo[*a*]pyrene 4,5-oxide	9.0 ± 1.4	22.5 ± 3.7	2.5	9.65 ± 1.5	18.5 ± 1.2	1.9
NADPH-cytochrome *c* oxidoreductase	0.314 ± 0.036	0.301 ± 0.008	1.0	0.342 ± 0.066	0.602 ± 0.068	1.8
UDP-glucuronosyltransferase	172 ± 10	242 ± 14	1.4	60 ± 9.0	161 ± 9.2	2.7
Cytochrome P-450	0.859 ± 0.130	1.58 ± 0.220	1.8	0.956 ± 0.031	2.78 ± 0.57	2.9
Cytochrome b_5	0.421 ± 0.027	0.565 ± 0.022	1.3	0.552 ± 0.024	0.677 ± 0.040	1.2

[a] Values represent the mean ± standard deviation from three separate experiments (three rats per experiment).

[b] Values are expressed as nanomoles per minute per milligram of protein except in the case of cytochromes P-450 and b_5, which are given in nanomoles per milligram of protein.

TABLE 2

Effects of trans-stilbene oxide on epoxide hydratase, NADPH-cytochrome c oxidoreductase, UDP-glucuronosyltransferase, cytochrome b_5, and cytochrome P-450 contents in rough and smooth microsomal membrane[a]

Enzyme	Specificy activity[b]					
	Rough microsomal membrane			Smooth microsomal membrane		
	Control	Induced	Induced/control	Control	Induced	Induced/control
Epoxide hydratase						
Styrene oxide	27.6 ± 2.1	95.2 ± 3.5	3.4	34.9 ± 1.7	93.6 ± 7.7	2.7
Benzo[*a*]pyrene 4,5-oxide	9.1 ± 1.8	31.7 ± 6.5	3.5	10.1 ± 0.5	31.4 ± 1.8	3.1
NADPH-cytochrome *c* oxidoreductase	0.341 ± 0.016	0.543 ± 0.024	1.6	0.387 ± 0.034	0.635 ± 0.055	1.6
UDP-glucuronosyltransferase	176 ± 10	173 ± 4	1.0	67.6 ± 3	160 ± 9	2.4
Cytochrome P-450	0.913 ± 0.150	1.63 ± 0.170	1.8	0.995 ± 0.060	1.99 ± 0.04	1.9
Cytochrome b_5	0.429 ± 0.030	0.563 ± 0.036	1.3	0.568 ± 0.020	0.619 ± 0.07	1.1

[a] Values represent the mean ± standard deviation for three separate experiments (three rats per experiment).

[b] Values are expressed as nanomoles per minute per milligram of protein except in the case of cytochrome P-450 and b_5, which are given in nanomoles per milligram of protein.

contrast, *trans*-stilbene oxide increased levels of the cytochrome approximately 2-fold in both membranes. Variations in the extent of induction of UDP-glucuronosyltransferase and NADPH-cytochrome *c* oxidoreductase were also noted between rough and smooth microsomes. Phenobarbital and *trans*-stilbene oxide induced transferase activity 2- to 3-fold in smooth microsomes, whereas rough microsomes remained near the control levels. Another interesting difference was noted between the inducibility of rough and smooth microsomes with respect to NADPH-cytochrome *c* oxidoreductase activity. Phenobarbital induced oxidoreductase activity to 1.8-fold above the control level in smooth microsomes but did not increase this activity in rough microsomes. *trans*-Stilbene oxide, however, marginally increased oxidoreductase activity to 1.6-fold above the control value in both membrane fractions. Only a slight increase in cytochrome b_5 was noted in either rough or smooth microsomes upon administration of *trans*-stilbene oxide or phenobarbital.

Effects of trans-stilbene oxide and phenobarbital on nuclear envelope-associated activities. Examination of epoxide hydratase activity in nuclear envelope isolated from rats administered either *trans*-stilbene oxide or phenobarbital revealed a striking difference (Table 3). Epoxide hydratase activity toward both styrene oxide and benzo[*a*]pyrene 4,5-oxide was completely refractory to induction by phenobarbital, with even a slight decrease in activity toward styrene oxide noted. *trans*-Stilbene oxide, however, dramatically increased levels of activity toward both substrates to 3.1-fold (styrene oxide) and 2.4-fold (benzo[*a*]pyrene 4,5-oxide) above control levels. This difference in inducibility was also noted when nuclei were examined (Table 4). In addition, nuclear envelope UDP-glucuronosyltransferase, cytochrome P-450, and cytochrome b_5 were marginally induced by *trans*-stilbene oxide but not induced by phenobarbital (Table 3); however, oxidoreductase activity was not increased by either inducing agent.

Electrophoretic and immunochemical analysis of epoxide hydratase levels in trans-stilbene oxide- and phenobarbital-induced nuclear envelope. Nuclear envelope was examined for absolute levels of the epoxide hydratase polypeptide in order to determine whether it

TABLE 3

Effects of phenobarbital and trans-stilbene oxide on NADPH-cytochrome c oxidoreductase, UDP-glucuronosyltransferase, cytochrome b_5, and cytochrome P-450 contents in nuclear envelope[a]

Enzyme	Specific activity[b]					
	trans-Stilbene oxide			Phenobarbital		
	Control nuclear envelope	Induced nuclear envelope	Induced/control	Control nuclear envelope	Induced nuclear envelope	Induced/control
Epoxide hydratase						
Styrene oxide	10.6 ± 0.6	32.6 ± 4.0	3.1	10.4 ± 0.5	7.53 ± 0.58	0.7
Benzo[*a*]pyrene 4,5-oxide	4.74 ± 0.39	11.5 ± 0.7	2.4	4.91 ± 0.50	5.47 ± 0.14	1.1
NADPH-cytochrome *c* oxidoreductase	0.108 ± 0.021	0.118 ± 0.020	1.1	0.109 ± 0.010	0.124 ± 0.015	1.1
UDP-glucuronosyltransferase	56.9 ± 4.0	79.4 ± 9.0	1.4	56.6 ± 5.0	52.9 ± 3.0	0.9
Cytochrome P-450	0.186 ± 0.033	0.253 ± 0.056	1.4	0.204 ± 0.040	0.225 ± 0.050	1.1
Cytochrome b_5	0.152 ± 0.024	0.217 ± 0.025	1.4	0.145 ± 0.030	0.160 ± 0.040	1.1

[a] Values represent the mean ± standard deviation for three separate experiments (15 rats per experiment).

[b] Values are expressed as nanomoles per minute per milligram of protein except in the case of cytochrome P-450 and b_5, which are given in nanomoles per milligram of protein.

TABLE 4

Effects of phenobarbital and trans-stilbene oxide on epoxide hydratase content in nuclei[a]

Epoxide hydratase	Specific activity[b]					
	trans-Stilbene oxide			Phenobarbital		
	Control nuclei	Induced nuclei	Induced/control	Control nuclei	Induced nuclei	Induced/control
Styrene oxide	0.625 ± 0.130	1.96 ± 0.150	3.1	0.629 ± 0.060	0.481 ± 0.020	0.8
Benzo[*a*]pyrene 4,5-oxide	0.630 ± 0.018	1.63 ± 0.180	2.6	0.627 ± 0.018	0.650 ± 0.044	1.0

[a] Values represent the mean ± standard deviation for three separate experiments (15 rats per experiment).

[b] Values are expressed as nanomoles per minute per milligram of protein.

corresponds to the differences in enzymatic activities obtained in induced and noninduced membranes. Electrophoretic analysis (Fig. 1) of *trans*-stilbene oxide- and phenobarbital-induced nuclear envelope clearly shows that the Coomassie blue-stained polypeptide, which comigrates with purified epoxide hydratase (Fig. 1, *Lanes 1* and *5*), is elevated in *trans*-stilbene oxide nuclear envelope (*Lane 2*) when compared with corn oil controls

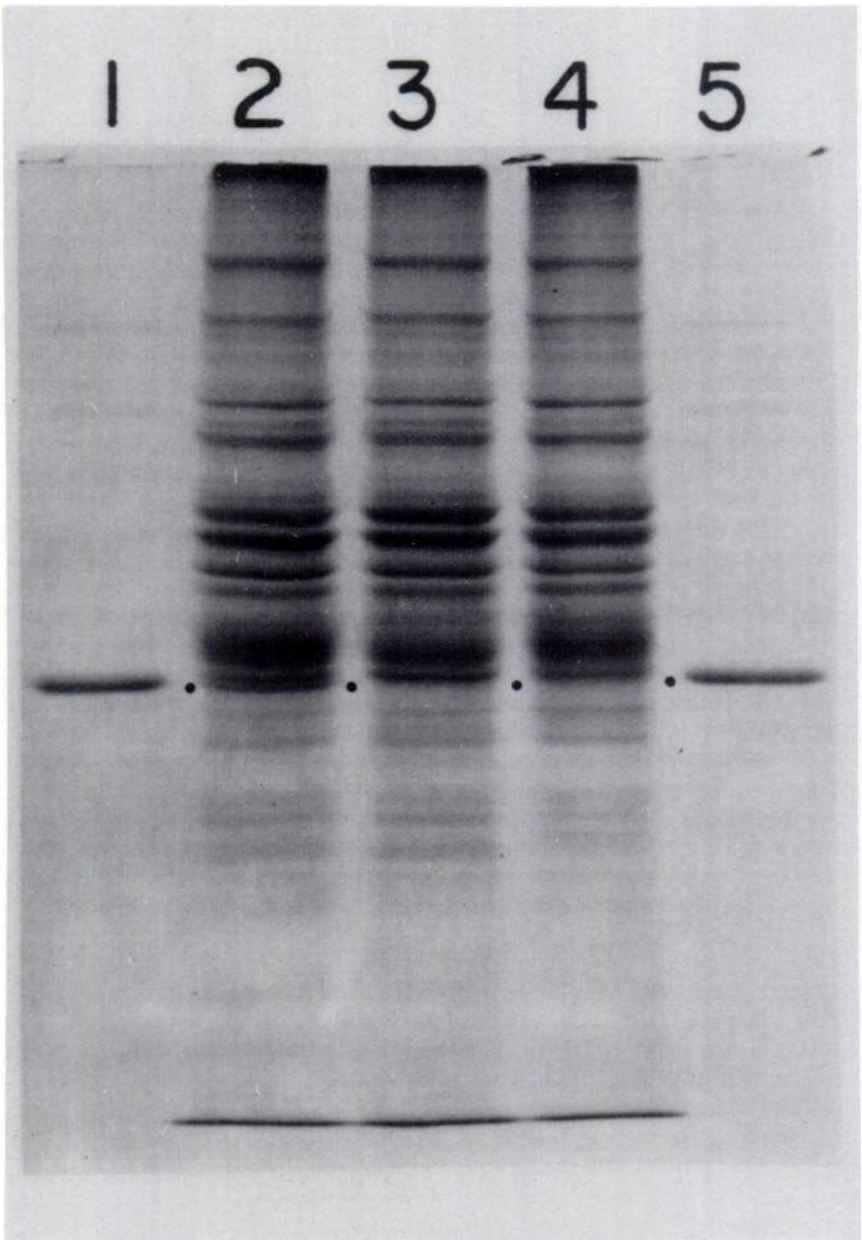

FIG. 1. *Electrophoretic analysis of nuclear envelope polypeptides isolated from trans-stilbene oxide-induced (Lane 2), control (Lane 3) and phenobarbital-induced (Lane 4) animals*

Nuclear envelope was isolated as described under Materials and Methods, solubilized, and electrophoresed on a sodium dodecyl sulfate-polyacrylamide gel (13). *Lanes 1* and *5* contain purified epoxide hydratase.

TABLE 5

Immunological quantitation of epoxide hydratase in trans-stilbene oxide and phenobarbital-induced microsomes and nuclear envelope

Membrane	% Epoxide hydratase[a]	Induced /control		
		Immunochemical quantitation	Enzymatic activity[b]	
			Benzo[a]pyrene 4,5-oxide	Styrene oxide
Smooth microsomes				
Control	2.13			
trans-Stilbene oxide	7.60	3.6	3.1	2.7
Phenobarbital	6.42	2.9	1.9	2.6
Rough microsomes				
Control	1.96			
trans-Stilbene oxide	8.08	4.1	3.7	3.4
Phenobarbital	5.34	2.7	2.5	2.2
Nuclear envelope				
Control	0.79			
trans-Stilbene oxide	2.41	3.1	2.4	3.1
Phenobarbital	0.96	1.2	1.1	0.7

[a] The percentage of epoxide hydratase is (amount of epoxide hydratase determined by radial immunodiffusion)/total membrane protein as assayed by the Folin method, ref. 4) ×100. Each value represents the average of triplicate determinations on a single membrane preparation.
[b] Taken from Tables 1–3.

(*Lane 3*), whereas the intensity of this band in phenobarbital nuclear envelope (*Lane 4*) is similar to control. The electrophoretic pattern of envelope purified from animals given injections of corn oil was identical with that from animals administered 0.15 M NaCl.

Radial immunodiffusion of epoxide hydratase (Table 5) revealed that the changes in epoxide hydratase activity upon induction by *trans*-stilbene oxide correlated with the changes in amounts of immunochemically titratable enzyme. Especially noteworthy is the lack of induction in nuclear envelope from phenobarbital-treated animals (1.2-fold) in contrast to that of phenobarbital-induced rough (2.7-fold) and smooth microsomes (3.0-fold). These results confirm those obtained using the enzyme assays.

DISCUSSION

The nuclear envelope mixed-function oxidase system and epoxide hydratase are involved in the metabolism of a variety of internally generated substrates as well as foreign compounds taken in from the environment. The fact that these enzymes are responsible for the metabolic activation of potentially carcinogenic compounds (32) emphasizes the importance of understanding their regulation.

The nuclear surface serves as a final site to hydrate carcinogenic or mutagenic epoxides formed by the mixed-function oxidase system, before they can come into contact with critical target molecules within the nucleus. Indeed, administration of *trans*-stilbene oxide, an inducer of epoxide hydratase, has been shown to decrease the mutagenicity of benzo[a]pyrene 4,5-oxide (33); hence, induction of this enzyme in the nuclear envelope may serve a vital role in protecting the genetic elements of the cell, whose modification could lead to transformation. In contrast, the enzyme also plays a role in the formation of the highly carcinogenic benzo[a]pyrene 7,8,-dihydrodiol 9,10-oxide (2, 32).

Evidence has been presented that suggests that both epoxide hydratase and cytochrome P-450 are also localized inside the nucleus (34). However, calculations based on the recovery of nuclear envelope and the data presented in Tables 3 and 4 reveal that 80–100% of nuclear epoxide hydratase activity (toward styrene oxide and benzo[a]pyrene 4,5-oxide) is recovered in the envelope. Hence, from a quantitative standpoint, nuclear envelope epoxide hydratase accounts for essentially all of the nuclear activity.

We have analyzed phenobarbital-induced nuclear envelope for epoxide hydratase and found no significant difference from control values, whereas *trans*-stilbene oxide dramatically increases the nuclear envelope enzyme. However, both compounds increase levels of epoxide hydratase associated with rough and smooth microsomes, and others have reported that phenobarbital does induce epoxide hydratase associated with nuclei and nuclear envelope (35, 36). The discrepancy between these results and ours is not clear; however, it may result from variations in the procedures used in the preparation of nuclei and nuclear envelope or differences in the assay methods. Our analyses performed on nuclear envelopes from phenobarbital-treated animals did not show elevated levels of immunoprecipitable epoxide hydratase and were in agreement with the specific activity measurements demonstrating no increased activity toward either benzo[a]pyrene 4,5-oxide or styrene oxide. Furthermore, electrophoretic analysis of nuclear envelopes from induced animals provides additional evidence supporting the observation that phenobarbital does not produce a measurable increase in envelope epoxide hydratase. The differential effects of *trans*-stilbene oxide and phenobarbital on the nuclear envelope enzyme are especially intriguing, since both agents appear to show similar patterns of induction in the endoplasmic reticulum.

Interestingly, NADPH-cytochrome *c* oxidoreductase and UDP-glucuronosyltransferase levels are not elevated in rough microsomes even though both enzymes are readily induced in smooth microsomes upon phenobarbital administration. *trans*-Stilbene oxide also preferentially increases transferase in smooth membrane. However, epoxide hydratase is found elevated in both membrane fractions after either phenobarbital or *trans*-stilbene oxide administration. These data indicate that the rough and smooth endoplasmic reticulum differ in their ability to accumulate various enzymes.

Recently, we found that the levels of NADPH-cytochrome *c* oxidoreductase and epoxide hydratase polysomal mRNAs are increased on the nuclear envelope soon after administration of phenobarbital (37). Induced levels of these mRNAs associated with the nuclear envelope rapidly decline concomitantly with their accumulation in rapidly sedimenting endoplasmic reticulum. Hence, at later times after a single dose of phenobarbital, the envelope does not contain elevated levels of epoxide hydratase or oxidoreductase mRNA, whereas these mRNAs remain elevated at a level 4-fold above the control value in rapidly sedimenting endoplasmic reticulum (37). Failure to maintain elevated levels of epoxide hydratase and oxidoreductase mRNAs associated with the nuclear envelope after phenobarbital administration

may account for the lack of induction of these enzymes in the envelope.

Currently, epoxide hydratase cDNA (38) is being used to study the molecular basis underlying the differential inductive effect of phenobarbital and *trans*-stilbene oxide on nuclear envelope epoxide hydratase. Furthermore, these two inducing agents provide a unique and valuable tool with which to elucidate events responsible for the biogenesis of intracellular membranes and the synthesis, turnover, and regulation of nuclear envelope-associated enzymes.

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