MOLECULAR PHARMACOLOGY, 15, 192-196

SHORT COMMUNICATION

Rat Hepatic Nuclear Cytochrome P-450 and Epoxide Hydrase in Membranes Prepared by Two Methods: Similarities with the Microsomal Enzymes

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(Received August 3, 1978)
(Accepted August 10, 1978)

SUMMARY

MUKHTAR, HASAN, ELMAMLOUK, TAHANI H., PHILPOT, RICHARD M. & BEND, JOHN R. (1979) Rat hepatic nuclear cytochrome P-450 and epoxide hydrase in membranes prepared by two methods: Similarities with the microsomal enzymes. *Mol. Pharmacol.* **15**, 192-196.

Cytochrome P-450 in rat liver nuclear membranes of untreated, PB-pretreated, or 3-MC-pretreated animals was spectrally similar to corresponding liver microsomes. Epoxide hydrase activity of liver nuclear membrane and microsomes was induced by PB administration; 3-MC did not cause any induction. The same results were obtained when either the DNase digestion method or sucrose density centrifugation technique for preparation of nuclear membranes was used.

INTRODUCTION

Cytochrome P-450 is the terminal oxidase of the monooxygenase system which is located primarily in the endoplasmic reticulum of the liver and several other tissues. This system is capable of metabolizing a variety of xenobiotics (1). The spectral characteristics of cytochrome P-450 and the relative rates of metabolism of several substrates have been used to characterize monooxygenase systems from both untreated animals and animals treated with various inducers (2-7).

Recent studies have shown that multiple forms of cytochrome P-450 are present in hepatic microsomes from animals treated with different inducing agents and in animals of different species treated with the same inducer. For example, hepatic microsomal cytochromes P-450 and P-448 purified from rats or rabbits treated with PB[2] or 3-MC, respectively, have different molecular weights and different catalytic, spectral, and immunological properties (2, 4, 5, 8-13).

Cytochrome P-450 has recently been identified in rat hepatic nuclei (14). Like its microsomal counterpart, nuclear cytochrome P-450 is induced by PB and 3-MC (14-17). Due to the potential importance of the monooxygenase system in the metabolic activation of carcinogens and mutagens, several laboratories (14-17), including our own (18), are investigating this enzyme system in the nucleus and in nuclear membrane preparations. One important question being asked concerns possible differences between the microsomal and nuclear forms of cytochrome P-450. Rogan and

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[2] The abbreviations used are: PB, phenobarbital; 3-MC, 3-methylcholanthrene.

0026-895X/79/010192-05$02.00/0

Cavalieri (19) have reported that cytochrome P-450 in rat hepatic nuclear membrane can be spectrally distinguished from the microsomal cytochrome. They reported an absorption maxima of 452 nm for nuclear cytochrome P-450 from control and PB-induced rats and 450 nm for the nuclear cytochrome from 3-MC-treated rats (19). The corresponding values obtained from microsomes are 450 and 448 nm. While Rogan and Cavalieri (19) have used nuclear membranes prepared by the method (digestion by treatment with DNase) of Kay *et al.* (20), we routinely assayed for cytochrome P-450 in nuclear membranes prepared by the method (sucrose density centrifugation) of Kashing and Kasper (21) and did not detect any spectral differences between the nuclear and microsomal cytochromes. In the present study we have examined the difference spectrum formed by the addition of carbon monoxide to dithionite-reduced cytochrome P-450 in nuclear membranes prepared by both methods.

Recently, epoxide hydrase, another important xenobiotic-metabolizing enzyme, has been identified in the nucleus (15, 22). We have shown that epoxide hydrase is associated with the nuclear membrane and that it can be induced with PB or *trans*-stilbene oxide (18, 23). The effect of the method used for the preparation of nuclear membranes on epoxide hydrase activity has also been examined.

Styrene oxide [8-^{14}C] (sp. act., 0.497 mCi/mmole; radiochemical purity > 98.5%) was purchased from New England Nuclear, Boston, Mass., and unlabeled styrene oxide was a product of Eastman Organic Chemicals, Rochester, N.Y. Benzo[*a*]pyrene 4,5-oxide[^{3}H] (sp. act., 10 mCi/mmole; radiochemical purity > 99%) and unlabeled benzo[*a*]pyrene 4,5-oxide were synthesized by Midwest Research Institute, Kansas City, Mo., under NCI contract No. N01-CP 33387 (supplemented by NIEHS) and further purified by chromatography on alumina columns in our laboratory prior to use. All other chemicals, of highest purity commercially available, were procured from sources described earlier.

Nuclei were isolated from livers (perfused *in situ* with ice-cold 0.25 M sucrose) of male Sprague-Dawley rats (100–150 g) as described earlier (17, 22). Isolated nuclei were washed four times in 1 M sucrose-1 mM $CaCl_2$. Nuclear membranes were prepared from washed nuclei by either the sucrose density centrifugation method of Kashing and Kasper (21) or the DNase digestion method of Kay *et al.* (20).

Rats were used untreated or following treatment with PB (80 mg/kg in corn oil on three consecutive days) or 3-MC (40 mg/kg in corn oil on two consecutive days) by i.p. injection. This treatment with 3-MC was optimal for the induction of hepatic microsomal cytochrome P-448 but did not induce epoxide hydrase activity. Hepatic microsomes were prepared by standard methods (24).

Protein was estimated according to Lowry *et al.* (25) using bovine serum albumin as a standard. The concentrations of microsomal and nuclear cytochrome P-450 were determined by the method of Omura and Sato (26) from membrane preparations suspended in 0.1 M Tris-20% glycerol, pH 7.4. An Aminco DW 2A spectrophotometer was used and maximum resolution was assured by recording the spectra in the fast response mode at a rate of 1 nm/sec. Protein concentrations below 3 mg/ml were used to avoid nonlinear responses. Contamination of the preparations with hemoglobin was determined spectrally (carbon monoxide minus oxidized difference spectrum) and was negligible in all samples used. Epoxide hydrase was determined by the thin-layer chromatographic procedure of Jerina *et al.* (27).

The difference spectra formed upon the addition of carbon monoxide to dithionite-reduced microsomes and nuclear membranes (prepared by the DNase-digestion method) from untreated and PB- and 3-MC-treated adult rats are shown in Fig. 1. The absorption maxima are 450 nm for the spectra of both the microsomal and nuclear cytochromes from untreated and PB-treated rats and 448 nm for the spectra of the microsomal and nuclear cytochromes from 3-MC-treated rats. The same results were obtained using nuclear membranes prepared by sucrose density centrifugation. The spectral maxima and cytochrome con-

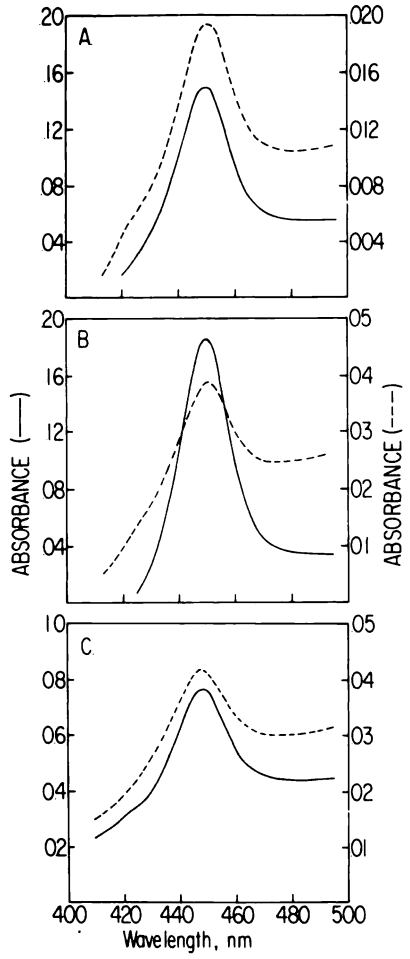

FIG. 1. *CO-difference spectra of dithionite reduced hepatic microsomes* (——) *and nuclear membranes* (-----)

A. Untreated animals. B. PB-pretreated animals. C. 3-MC-pretreated animals. Concentrations of protein (mg/ml) in the cuvette used were 0.97, 0.59, and 1.02 for A, B, and C microsomes, respectively, and 2.92, 1.91, and 1.52 for A, B, and C nuclear membranes.

centrations are shown in Table 1. (The exact wavelengths may deviate from the reported by ± 0.2 nm, but in no case was any detectable difference observed between the microsomal and nuclear spectra from rats treated in the same manner.) Both microsomal and nuclear cytochrome P-450 were induced by PB and 3-MC (Table 1). In other experiments (data not shown) where 3-MC was injected as a single dose (40 mg/kg) and animals were sacrificed 24 hr after treatment, we observed a partial shift in the wavelength of the cytochrome P-450 spectrum (from 450 ± 0.2 to 449 ± 0.2 nm). This partial shift in wavelength was identical in hepatic microsomes and nuclear membranes.

Epoxide hydrase activities in nuclear membranes isolated by either procedure were the same (Table 1). Both nuclear and microsomal epoxide hydrase activities were induced byPB but not by 3-MC. Again, the method of nuclear membrane preparation did not affect the results. The observed effects of PB and 3-MC on nuclear epoxide hydrase were consistent with our earlier findings with microsomal activity (28).

Our results show that rat hepatic nuclear and microsomal cytochrome P-450 cannot be distinguished on the basis of their carbon monoxide difference spectra and that the inductive effects of PB and 3-MC on microsomal and nuclear cytochrome P-450 and epoxide hydrase are similar. While this manuscript was in preparation, two abstracts were published which support our conclusion that rat hepatic microsomal and nuclear cytochrome P-450 are similar: Chung and Kicha (29) reported that the spectral characteristics and inducibility of microsomal and nuclear cytochrome P-450 are similar and Bresnick *et al.* (30) reported that nuclear cytochrome P-448 cross-reacts with the antibody to microsomal cytochrome P-448. In addition, using intact nuclei Bresnick *et al.* (17) have demonstrated a close similarity between nuclear and microsomal cytochrome P-450. The reasons for the discrepancies between the above results and those of Rogan and Cavalieri (19) are not clear, but could be attributed in part to the fact that they used 3-week-old immature rats whereas we have used 100–150 g body weight (5–6 weeks old) animals in the present study. However, we did not find any apparent shift in the wavelengths of the cytochrome P-450 of nuclear membrane between 3-, 4-, or 5-week old rats (data not presented). In addition, the method used for the preparation of nuclear membranes does not appear to be a relevant factor.

The similar characteristics of the hepatic microsomal and nuclear monooxygenase systems and epoxide hydrase suggest a common biogenesis. Moreover, the presence of significant monooxygenase and

TABLE 1

Cytochrome P-450 concentrations and epoxide hydrase activities in microsomal and nuclear membranes prepared from untreated and PB and 3-MC-treated rats

Carbon monoxide difference spectra were measured as described in the text. The protein concentration in the cuvette ranged from 0.59–2.92 mg/ml. Epoxide hydrase activity (with benzo(a)pyrene 4,5-oxide as substrate) was determined as described in text. Values given are mean ± SD with number of experiments in parentheses.

Treatment	Membrane preparation	Cytochrome P-450		Epoxide hydrase activity
		λ *max(mm)*[a]	*nmol/mg protein*	*(nmol product/min/mg protein)*
Control	Nuclear-SDC[b] method	450	0.032	0.98 ± 0.13 (6)
Control	Nuclear-DNase method	450	0.033	0.84 ± 0.10 (2)
Control	Microsomal	450	1.21	9.67 ± 0.84 (6)
PB	Nuclear-SDC method	450	0.079	2.40 ± 0.40 (2)
PB	Nuclear-DNase method	450	0.075	2.22 ± 0.31 (2)
PB	Microsomal	450	2.83	25.80 ± 1.23 (3)
3-MC	Nuclear-SDC method	448	0.081	1.01 ± 0.19 (2)
3-MC	Nuclear-DNase method	448	0.078	0.94 ± 0.16 (2)
3-MC	Microsomal	448	3.44	10.29 ± 0.98 (2)

[a] The exact wavelengths may deviate from these values by ± 0.2 nm but no detectable differences occurred between microsomal and nuclear membranes.

[b] SDC-Sucrose density centrifugation.

epoxide hydrase activities in the nucleus is consistent with a role for this organelle in the bioactivation of some environmental pollutants to carcinogenic and/or mutagenic products.

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MOLECULAR PHARMACOLOGY

Published bimonthly by Academic Press, Inc., 111 Fifth Avenue, New York, N. Y. 10003. Number of issues published annually: 6. Editor: Norman Kirshner, Duke University Medical Center, Durham, N. C. 27710.
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