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EXPRESSION OF MICROSOMAL AND CYTOSOLIC EPOXIDE HYDROLASES IN CULTURED RAT HEPATOCYTES AND HEPATOMA CELL LINES

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SUMMARY: Microsomal epoxide hydrolase activity, determined using benzpyrene 4,5-oxide and styrene 7,8-oxide, increased in cultured hepatocytes compared to freshly isolated cells. In contrast, cytosolic epoxide hydrolase activity, assayed using trans-stilbene oxide, had decreased 80% by 24 hr and was barely detectable after 96 hr in culture. There was no difference in enzyme activity between freshly isolated hepatocytes and the two rat hepatoma cell lines McA-RH 7777 and H4-II-E, when stryrene 7,8-oxide was used as substrate. However, benzpyrene 4,5-oxide hydrolase activity of the McA-RH 7777 and H4-II-E cell lines were 55 and 10%, respectively, of freshly isolated hepatocytes. These results show that hepatoma cell lines provide a suitable system for studying the regulation of both the microsomal and cytosolic epoxide hydrolase enzymes.

Epoxides of many xenobiotics are strong electrophiles capable of covalent interaction with critical cellular macromolecules (e.g. DNA), and have consequently been implicated in the mutagenic and carcinogenic effects of chemicals (1). The metabolic processing of these epoxides by epoxide hydrolase can lead either to metabolic activation or detoxification. Although the latter is generally the case, it has been shown that certain polycyclic aromatic hydrocarbon epoxides are converted by epoxide hydrolase to diols that are precursors for the bay region diol expoxides, currently thought to be the ultimate carcinogenic derivatives of these compounds (2). At least two distinct forms of epoxide hydrolase have been identified; a microsomal hydrolase and a cytosolic hydrolase (1,2). Evidence is now accummulating that there is more than one form of microsomal epoxide hydrolase (3-5) and a similar case may also exist for the cytosolic enzyme (6).

Many hepatocarcinogens have been shown to induce the activity of rat liver microsomal epoxide hydrolase(s) in the early stages of hepatocarcino-

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genesis and this enzyme(s) has consequently been proposed as a marker for preneoplastic hepatocytes (7-9). To gain further insight into the regulation of these enzymes we have studied their expression in both primary rat hepatocytes and rat hepatoma cell lines. Styrene 7,8-oxide and benzpyrene 4,5-oxide were used as probes for microsomal expoxide hydrolase(s), and trans-stilbene oxide for the cytosolic enzyme.

METHODS

Chemicals

[^{3}H]-Styrene 7,8-oxide (90 mCi/mmol) was purchased from Amersham (Arlington Heights, IL) and styrene 7,8-oxide from Matheson, Coleman & Bell (Los Angeles, CA). [^{3}H] Benzpyrene 4,5-oxide (342 mCi/mmol) and benzpyrene 4,5-oxide were obtained from the Chemical Carcinogen Reference Standard Repository, the Division of Cancer Cause and Prevention, NCI, NIH, Bethesda, MD. [^{3}H] Trans-stilbene oxide (69 mCi/mmol) was generously donated by Dr. B. D. Hammock, University of California, Riverside, CA. Unlabeled trans-stilbene oxide was obtained from Aldrich Chemical Company (Milwaukee, WI) and sodium cholate from Sigma Chemical Company (St. Louis, MO). All other chemicals were of the highest purity commercially available.

Animals, preparation of hepatocytes and cell culture

Male Sprague-Dawley rats (Taconic Farms, Germantown, NY) weighing 200-250 g, were provided with food and water ad libitum. Hepatocytes were prepared as previously described (10) and cultured in Waymouths medium supplemented by the following ingredients: $NaHCO_3$ (2.24 g/l), Hepes (3.57 g/l), L-alanine (11.2 mg/l), L-asparagine (24 mg/l), L-serine (12.8 mg/l), streptomycin (100 mg/l) and gentamycin (50 mg/l). In addition the following were added: insulin (8.3×10^{-7} M), dexamethasone (5×10^{-8} M), progesterone (1×10^{-6}M), testosterone (1×10^{-6} M), estradiol (5×10^{-7} M), glucagon (1×10^{-6} M), thyroxine (2.3×10^{-8} M), α-tocopherol (1.2×10^{-5} M), L-ascorbic acid (3×10^{-4} M), transferrin (6×10^{-8} M), linoleic acid (1.8×10^{-5} M) and δ-aminolevulenic acid (1×10^{-6} M). Three ml of hepatocytes (5×10^5 cells/ml) were seeded in plastic tissue culture dishes (60 mm) coated with collagen (Bovine Type I) and incubated at 37°C in a humidified incubator under 5% CO_2/95% air atmosphere. The medium was changed after 4 hours to remove unattached cells and then every 24 hours throughout the incubation period.

Hepatoma cell lines

Rat hepatoma cell lines McA-RH 7777 and Reuber H4-II-E were maintained as previously described (11,12). Cells were seeded in 75 cm^2 plastic flasks at a cell density of 3×10^5 cells/cm^2 containing 12 ml medium. The medium was changed every 24 hr and after 3 days of culturing cells were near confluent. The medium was then removed and the cells were washed twice with 5 ml of ice-cold phosphate-buffered saline (0.15 M NaCl/0.014 M KH_2PO_4/0.086 M K_2HPO_4), pH 7.4. An additional 5 ml of buffer was added and the cells were scraped free and sedimented by centrifugation at 300 g for 5 min. The supernatant was removed and the cell pellet was resuspended in 0.5 to 1 ml of 76 mM phosphate buffer, pH 7.4 and then disrupted by ultrasonification (Kontes, Vineland, NJ) for three 10-sec pulses at 1-min intervals. Cultured hepatocytes were dislodged in a similar manner and sedimented at 50 g for 3 min and both these and freshly isolated hepatocytes were disrupted by the above procedure. Protein was determined by the method of Lowry et al. (13) using bovine serum albumin as a standard. To avoid any modification of enzyme activity assays were conducted immediately after sonification.

Epoxide hydrolase assays

Radiometric extraction assays for the determination of epoxide hydrolase activity were performed as described using the following substrates: benzpyrene 4,5-oxide (14), styrene 7,8-oxide (15) and trans-stilbene oxide (16). The tritiated substrates were diluted with the corresponding unlabeled compounds to give final concentrations of 25 μM, 2 mM and 50 μM for benzypyrene 4,5-oxide, styrene 7,8-oxide and trans-stilbene oxide, respectively. Total cellular protein concentration ranged from 50 to 100 μg/ml and incubation times were 1, 5, and 2 min for benzpyrene 4,5-oxide, styrene 7,8-oxide and trans stilbene oxide, respectively as substrates. These conditions gave reaction rates that were zero order with respect to substrates and linear with respect to protein concentration and time.

RESULTS AND DISCUSSION

Fig. 1A shows the activity of microsomal epoxide hydrolase, measured using benzpyrene 4,5-oxide and styrene 7,8-oxide as substrates, in hepatocytes over 96 hr in culture. In freshly isolated hepatocytes microsomal epoxide

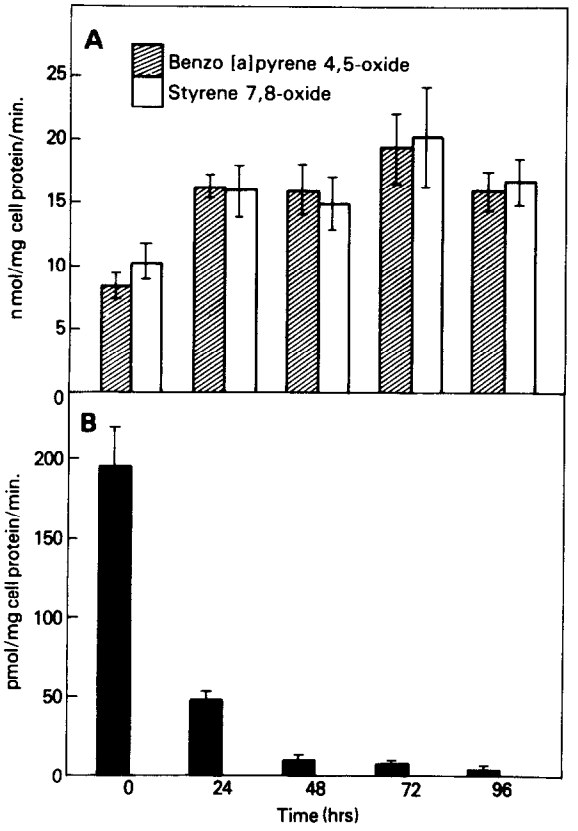

Figure 1. Effect of time in culture of rat hepatocytes on (A) microsomal epoxide hydrolase, measured using benzpyrene 4,5-oxide and styrene 7,8-oxide as substrates, (B) cytosolic epoxide hydrolase activity, measured using trans-stilbene oxide as the substrate.

hydrolase activity was 7.7 ± 0.8 and 10.8 ± 1.9 nmol/mg cellular protein/min (mean ± SD, n = 3) with benzpyrene 4,5-oxide and styrene 7,8 oxide as substrates, respectively. After 24 hr in culture, enzyme activity determined by both substrates had increased to greater than 50% of freshly isolated cells and remained elevated throughout the four days in culture. This is in marked contrast to the activity of cytochrome P-450 monooxygenases, an enzyme system that is responsible for the production of many xenobiotic epoxides, which undergoes a marked decrease in activity in cultured rat hepatocytes as compared to freshly isolated cells (17). The expression of microsomal epoxide hydrolase in cultured rat hepatocytes is similar to γ-glutamyl transpeptidase (18) which has also been proposed as a marker of preneoplastic cells. The significance of the increase in activity of these two enzymes in tissue culture and the relationship of this phenomenon to the preneoplastic state remains to be elucidated.

In marked contrast to the microsomal epoxide hydrolase activity, the cytosolic enzyme which showed an initial specific activity of 195 ± 25 pmol/mg cellular protein/min (mean ± SD, n = 3) with trans-stilbene oxide as a substrate in freshly isolated hepatocytes, decreased 80% after 24 hr in culture and was barely detectable at 96 hr (Fig. 1B).

Both microsomal and cytosolic epoxide hydrolase activities were readily detectable in whole cell homogenates from the McA-RH 7777 and Reuber H4-I hepatoma cell lines (Table I). There was no significant difference in enzyme

TABLE I. Epoxide Hydrolase Activity in Freshly Isolated Hepatocytes and Hepatoma Cell Lines.

Cell Type	Epoxide Hydrolase Activity		
	Benzpyrene Dihydrodiol[a]	Styrene Dihydrodiol[a]	Stilbene Dihydrodiol[b]
Hepatocytes	7.7 ± 0.8	10.3 ± 1.9	195 ± 25
McA-RH 7777	4.2 ± 0.7	9.1 ± 3.4	104 ± 21
H4-II-E	0.74 ± 0.04	10.1 ± 1.5	125 ± 22

Values represent mean ± SD, n = 3.
[a] Activity expressed as nmol/mg cellular protein/min
[b] Activity expressed as pmol/mg cellular protein/min

activity between freshly isolated hepatocytes and the two hepatoma cell lines when styrene 7,8-oxide was employed as a substrate for the microsomal enzyme. However, when benzpyrene 4,5-oxide was used as a substrate McA-RH 7777 and H4-II-E cells had 55 and 10%, respectively, of the activity of freshly isolated hepatocytes. Raphael et al. (19) have similarly reported a low epoxide hydrolase activity in sonicated H4-II-E cells using benzpyrene 4,5-oxide as a substrate. Unlike cultured hepatocytes the two hepatoma cell lines display high cytosolic epoxide hydrolase activity. The McA-RH 7777 and H4-II-E cells had 52 and 64% of the activity of freshly isolated hepatocytes, respectively (Table I).

The differential expression of microsomal epoxide hydrolase in the two hepatoma cell lines, when assayed using either benzpyrene 4,5-oxide or styrene 7,8-oxide, is suggestive of more than one form of this enzyme. Levin et al. (4) have recently shown that the form of microsomal epoxide hydrolase which catalyzes the conversion of cholesterol 5,6α-oxide to the 5,6-glycol is antigenetically distinct from the microsomal epoxide hydrolase that metabolizes a wide range of xenobiotic epoxides. Further evidence on the multiplicity of this enzyme comes from studies showing that various inhibitors, activators and inducing agents have a differential effect on microsomal xenobiotic epoxide hydrolase activity depending on the substrate used (2,20). However, the most convincing evidence of the multiplicity of microsomal epoxide hydrolase comes from the work of Guengerich et al. (3,4) who have separated different forms of this enzyme by column chromatography. It should be pointed out, however, that the different forms of epoxide hydrolase separated by these authors were immunologically similar and had identical minimum molecular weights in SDS polyacrylamide gels, displayed similar peptide maps following trypsin digestion, and possessed overlapping substrate specificities.

The present study clearly shows that microsommal and cytosolic epoxide hydrolase enzymes are under distinctly different regulatory control. Further, the hepatoma cell lines used appear to provide a suitable system for studying the regulation of the microsomal and cytosolic epoxide hydrolase enzymes.

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