Induction of 5-aminolaevulinate synthase by two- to five-carbon alcohols in cultured chick-embryo hepatocytes

Relationship to induction of cytochrome *P*-450

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The induction of 5-aminolaevulinate synthase and of cytochrome *P*-450 by short-chain aliphatic alcohols was compared in primary cultures of chicken-embryo hepatocytes. Isopropyl alcohol, isobutanol, pentan-1-ol and isopentanol alone caused up to a 4-fold increase in 5-aminolaevulinate synthase, whereas ethanol and propan-1-ol did not. Induction of the synthase by isopentanol was maximal at 8 h, and reached a plateau thereafter, whereas the activity induced by 2-propyl-2-isopropylacetamide continued to increase for 20 h. In the presence of 3,4,3',4'-tetrachlorobiphenyl, an inhibitor of haem synthesis at the uroporphyrinogen decarboxylase step, synergistic induction of 5-aminolaevulinate synthase was observed with all the alcohols except ethanol. Ethanol, but not isopentanol, decreased the extent of induction of 5-aminolaevulinate synthase by 2-propyl-2-isopropylacetamide and 3,4,3',4'-tetrachlorobiphenyl (50% decrease at 112 mM-ethanol). Total protein synthesis was not inhibited by ethanol in these cells. The composition of porphyrins was determined after treatment of cells with ethanol, isopentanol or 2-propyl-2-isopropylacetamide. Untreated cells, when incubated with 5-aminolaevulinate for 6 h, accumulated mainly protoporphyrin. However, when cells were pretreated with ethanol, isopentanol or 2-propyl-2-isopropylacetamide for 20 h, and 5-aminolaevulinate was added, 8- and 7-carboxyporphyrins increased, whereas protoporphyrin decreased. The dose responses for induction of either 5-aminolaevulinate synthase or cytochrome *P*-450 after a 20 h exposure to 3- to 5-carbon alcohols were identical. The results indicate that: (1) simple alcohols can induce both enzymes; (2) hydrophobicity increases their effectiveness; and (3) induction of both enzymes are probably mediated by a common mechanism.

INTRODUCTION

A variety of chemicals induce 5-aminolaevulinate synthase and porphyrin accumulation in primary cultures of chicken-embryo hepatocytes (for review, see Marks, 1978). In this system a common property shared by inducing chemicals is hydrophobicity (De Matteis, 1978; Marks, 1978). Other factors suggested to increase the porphyrinogenicity of chemicals include resistance to metabolism and electron-donating properties of substituents (Marks, 1978).

Most chemicals that induce hepatic 5-aminolaevulinate synthase, the first and rate-limiting enzyme in the haem-biosynthetic pathway, also induce hepatic cytochrome *P*-450 (for a review, see Bonkowsky, 1982). Several hypotheses have been suggested to explain the interrelationship between induction of cytochrome *P*-450 and of 5-aminolaevulinate synthase, but no conclusive mechanism has been identified (for a review, see De Matteis, 1978).

We have reported that C_2 – C_5 alcohols cause a phenobarbital-like induction of cytochrome *P*-450 in cultured chick-embryo hepatocytes (J. Sinclair *et al.*, 1982a; J. Sinclair *et al.*, 1984). Characteristics of this

induction include (a) increases in (i) hydroxylation of biphenyl at the 4- rather than the 2-position, (ii) UDP-glucuronyltransferase activity, (iii) demethylation of benzphetamine and (b) little to no de-ethylation of ethoxyresorufin. A quantitative analysis of structure-activity relationships demonstrated a linear correlation between induction of cytochrome *P*-450 and hydrophobicity of the alcohols (Sinclair *et al.*, 1986). Here we investigated whether 2- to 5-carbon-chain alcohols also induce 5-aminolaevulinate synthase. In dose response studies with C_3 – C_5 alcohols, a direct correlation was found between induction of 5-aminolaevulinate synthase and cytochrome *P*-450. These alcohols are the simplest molecules yet reported to induce both hepatic responses, and therefore may be useful in elucidating the mechanism of the co-ordinated induction of these enzymes.

MATERIALS AND METHODS

Materials

3,4,3',4'-Tetrachlorobiphenyl (TCB) was purchased from Ultrascience, Hope, RI, U.S.A.; ethanol (100%,

Abbreviation used: TCB, 3,4,3',4'-tetrachlorobiphenyl.

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v/v; USP grade) was from the U.S. Industrial Chemical Company, Tuscola, IL, U.S.A.; C₃–C₅ alcohols were from Fisher Scientific Co., Fair Lawn, NJ, U.S.A. (all alcohols except ethanol were glass-distilled before use); Williams E medium was from Flow Laboratories, McLean, VA, U.S.A.; Hepes was from Calbiochem–Behring Corp., La Jolla, CA, U.S.A.; dexamethasone phosphate was from Lemmon Co., Settersville, PA, U.S.A.; 3,3',5-tri-iodothyronine and bovine serum albumin were from Sigma, St. Louis, MO, U.S.A.; 2-propyl-2-isopropylacetamide was a gift from Hoffman–La Roche, Nutley, NJ, U.S.A. The marker porphyrins octa-, hepta-, hexa-, penta- and tetra-carboxyporphyrins and mesoporphyrin were obtained from Porphyrin Products, Logan, UT, U.S.A.

Hepatocyte cultures

Primary cultures from livers of 17-day White-Leghorn-chicken embryos were prepared and maintained in Williams E medium as described previously (J. Sinclair *et al.*, 1982b). Cells were cultured in either 6 cm or 10 cm diameter culture dishes at 15% or 30% saturation with cells as indicated in the legend. Low cell density was found optimal for chemical induction of 5-aminolaevulinate synthase (Sinclair & Granick, 1977). At 20 h after plating the cells, the medium was changed to Williams E containing dexamethasone (0.3 µg/ml), 3,3',5-tri-iodothyronine (1 µg/ml) and 20 mM-Hepes buffer, pH 7.4. All plates containing alcohols were wrapped in Parafilm and Reynolds 912 film, a procedure found to minimize loss of ethanol (Sinclair *et al.*, 1981). The duration and specific type of treatment are given in the legends.

Assays

5-Aminolaevulinate synthase was measured by the procedure of Sinclair & Granick (1977) in either cell homogenates prepared by 11 strokes of a Potter–Elvehjem homogenizer or sonicated samples (Heat Systems Ultrasonix sonifier cell disruptor model W140D equipped with a microtip; samples treated for 3 s at position 2).

To measure low levels of the enzyme, cells from two 10 cm dishes (0.6–1.2 mg protein/dish) were harvested together in 0.3 ml total volume to provide approx. 1.0 mg protein per assay. With this amount of protein, when the spectrum of the pyrrole formed from 5-aminolaevulinate was scanned from 650 to 500 nm (on a Bausch and Lomb Spectronic 2000 spectrophotometer), the change in absorbance from 600 nm to the peak at 554 nm was approx. 0.005 absorbance units. 5-Aminolaevulinate synthase activity in homogenates or 'sonicates' untreated cells from eight separate experiments was 0.22 ± 0.05 nmol of 5-aminolaevulinate/30 min per mg of protein, activities within the range reported by other researchers for untreated cultured chick-embryo hepatocytes (Sassa & Kappas, 1977; Marks, 1978; Giger & Meyer, 1980).

Cytochrome *P*-450 was measured in the 8700 *g* supernatants prepared from cell homogenates as described previously (Sinclair *et al.*, 1979).

Porphyrins were analysed either spectrofluorometrically or by h.p.l.c. In the spectrofluorometric analysis, cells and media were harvested together and sonicated for 3 s at position 2 with the sonifier cell disruptor. A portion was mixed with an equal volume of 0.6 M-HClO₄/methanol (1:1, v/v) and porphyrins measured in the extract after centrifugation at 1000 *g* for 10 min. A modification of the method of Grandchamp *et al.* (1982) was used to quantify

the porphyrins spectrofluorometrically using a Perkin–Elmer Hitachi 512 Spectrofluorimeter (P. Sinclair *et al.*, 1984a). For analysis of porphyrins by h.p.l.c., the following modification of the procedure of Ford *et al.* (1981) was used. Cells and media were harvested together and sonicated. A portion was mixed with an equal volume of conc. HCl/acetone (1:39, v/v) and the mixture was centrifuged at 1000 *g* for 10 min. A 50 µl portion of the supernatant adjusted to pH 3–5 was injected into a Waters high-pressure liquid chromatograph (Waters Associates, Milford, MA, U.S.A.) and the porphyrins separated by using the following conditions; column, 5 µ C₁₈ Waters Radialpak; solvents: A, methanol/0.1 M-ammonium phosphate (11:14, v/v), pH 3.5; B, 100% methanol; convex gradient, 30%–100% B over 15 min, at a flow rate of 1.5 ml/min; fluorescence was detected on a Shimadzu Fluorescence h.p.l.c. monitor RF 530 (398 nm excitation, 620 nm emission) (Shimadzu Instrument Co., Columbia, MD, U.S.A.) interfaced to a Shimadzu C-R3A integrator. Appropriate standards were run for both quantification and identification of the individual porphyrins. Recoveries of standards added to cell cultures were $110 \pm 10\%$ (means $\pm$ s.d.) for 8- to 2-carboxyporphyrins.

Proteins were measured by the procedure of Lowry *et al.* (1951), with bovine serum albumin as a standard.

Protein synthesis was determined by the incorporation of [¹⁴C]leucine into acid-insoluble material; the medium from a 10 cm dish was decreased to a volume of 5 ml and [¹⁴C]leucine (300 mCi/mmol, New England Nuclear, MA, U.S.A.) added to a final concentration of 0.5 µCi/ml. After incubation at 37 °C for 1 h, the labelling was arrested by the addition of trichloroacetic acid (5%, w/v, final concn). After 10 min at room temperature, the fixed-cell layer was washed three times with saline (0.9% NaCl; 5 ml/wash) and harvested in 0.2 M-NaOH containing 1% (w/v) SDS. The dissolved material was neutralized with conc. HCl before adding the scintillation fluid (Aqueous Counting Scintillant; Amersham/Searle, Des Plaines, IL, U.S.A.).

RESULTS

Effect of C₂–C₅ alcohols on the activity of 5-aminolaevulinate synthase

As shown in Table 1, C₃–C₅ alcohols caused increases in 5-aminolaevulinate synthase activity when cultured hepatocytes were exposed to these alcohols at concentrations previously shown to yield maximal amounts of cytochrome *P*-450 (J. Sinclair *et al.*, 1982a; 1984). Ethanol and propan-1-ol, however, did not induce 5-aminolaevulinate synthase under these conditions. The maximal activities of 5-aminolaevulinate synthase induced by any of the alcohols were lower than those observed after treatment of cells with 2-propyl-2-isopropylacetamide, an inducer of 5-aminolaevulinate synthase in this system (Krupa *et al.*, 1974). TCB at a concentration (0.86 µM) found to inhibit haem synthesis 60% with accumulation of uroporphyrin (P. Sinclair *et al.*, 1984a) caused a synergistic induction of 5-aminolaevulinate synthase by both C₃–C₅ alcohols and 2-propyl-2-isopropylacetamide. The ability of the C₃–C₅ alcohols to induce 5-aminolaevulinate synthase, in both the presence and absence of TCB, increased with increasing hydrophobicity of the alcohols.

Table 1. Effect of C₂–C₅ alcohols on the induction of 5-aminolaevulinate synthase activity

Cells were plated on 10 cm dishes at approx. 0.6 mg of protein/dish and exposed to the chemicals as described in the Materials and methods section. The doses used were those shown in previous dose–response studies to cause maximal induction of cytochrome *P*-450 (Sinclair *et al.*, 1982a; 1984). 5-Aminolaevulinate synthase was measured in cell homogenates according to the colorimetric assay of Sinclair & Granick (1977). Each value represents the mean of duplicate samples with the individual values given in parentheses. Cells that were exposed to alcohols in the absence of TCB were analysed for porphyrins spectrofluorimetrically as described in the Materials and methods section. Each porphyrin value represents the mean for duplicate treatments with the individual values varying no more than 10%. The results represent two separate experiments, one with and one without the inclusion of 0.86 μ M-TCB.

Compound	Concn. (mM)	Total porphyrins (–TCB) (nmol/dish)	5-Aminolaevulinate synthase activity (nmol of 5-aminolaevulinate/30 min per mg of protein)	
			– TCB	+ TCB
None	–	0.22	0.23 (0.21, 0.24)	1.5 (1.4, 1.5)
Ethanol	200	0.25	0.18 (0.14, 0.23)	1.6 (1.5, 1.6)
Propan-1-ol	50	0.25	0.22 (0.19, 0.24)	2.3 (2.2, 2.4)
Propan-2-ol	100	0.39	0.46 (0.44, 0.48)	2.5 (2.5, 2.5)
Butanol	20	0.42	0.85 (0.7, 1.0)	6.8 (6.8, 6.9)
Isobutanol	30	0.73	0.47 (0.45, 0.50)	7.2 (6.7, 7.8)
Pentan-1-ol	10	1.29	0.50 (0.40, 0.60)	8.6 (7.6, 9.6)
Isopentanol	10	2.32	0.95 (0.90, 1.0)	10.8 (10.7, 10.8)
2-Propyl-2-isopropylacetamide	0.14	2.29	7.0 (6.4, 7.6)	21.1 (20.0, 22.3)

Table 2. Effects of piperonyl butoxide on porphyrin accumulation from 5-aminolaevulinate

Cells were pretreated with piperonyl butoxide (30 μ M) where indicated for 1 h. 5-Aminolaevulinate was then added along with the other chemicals listed and incubation continued for an additional 6 h. Cells and media were harvested together, sonicated, and porphyrins analysed by h.p.l.c. as described in the Materials and methods section. Each value represents the mean $\pm$ s.d. for triplicate dishes. Total porphyrin levels (nmol/dish): – piperonyl butoxide, 1.15 $\pm$ 0.14; + piperonyl butoxide, 1.02 $\pm$ 0.05

Additional treatment	[Piperonyl butoxide] (μ M)	Porphyrins accumulated (nmol/dish)		
		8- to 5-Carboxy-	Coproporphyrin	Protoporphyrin
None	0	0.14 $\pm$ 0.04	0.27 $\pm$ 0	0.57 $\pm$ 0.06
	30	0.07 $\pm$ 0	0.38 $\pm$ 0.01	0.54 $\pm$ 0.02
Ethanol	0	0.39 $\pm$ 0.06	0.44 $\pm$ 0.02	0.29 $\pm$ 0.06
	30	0.05 $\pm$ 0	0.54 $\pm$ 0.04	0.45 $\pm$ 0.02
Isopentanol	0	0.45 $\pm$ 0.06	0.22 $\pm$ 0.01	0.50 $\pm$ 0.05
	30	0.02 $\pm$ 0	0.24 $\pm$ 0.01	0.71 $\pm$ 0.09
2-Propyl-2-isopropylacetamide	0	0.63 $\pm$ 0.13	0.25 $\pm$ 0.01	0.45 $\pm$ 0.05
	30	0.06 $\pm$ 0.01	0.34 $\pm$ 0.02	0.66 $\pm$ 0.03

Porphyrin accumulation

Total accumulated porphyrin levels were similar in cells treated with either isopentanol or 2-propyl-2-isopropylacetamide, even though 5-aminolaevulinate synthase activities were 7-fold greater in the latter treatment (Table 1). Such lack of correlation between levels of 5-aminolaevulinate synthase and accumulated porphyrins has also been found by other investigators (Creighton & Marks, 1972; Taub & Marks, 1973; Sinclair & Granick,

1977). Previously, others reported that ethanol causes inhibition of uroporphyrinogen decarboxylase in the liver of rats (Doss *et al.*, 1981) and increased urinary excretion of coproporphyrin in humans (Sutherland & Watson, 1951; McColl *et al.*, 1980).

We have previously demonstrated that generation of porphyrins by intact cells from exogenous 5-aminolaevulinate was a sensitive method for detecting alterations in the pathway of porphyrin biosynthesis (Sinclair *et al.*, 1983). After incubation with exogenous 5-aminolaev-

Fig. 1. Composition of porphyrins generated from exogenous 5-aminolaevulinate

Cells were plated on 6 cm dishes and exposed to the various chemicals for 20 h. At the end of this time the volume of media was decreased to 1.5 ml/dish, and 5-aminolaevulinate was added to a final concentration of 150 μ M. After 6 h incubation, the dishes were chilled, cells and media harvested and porphyrins analysed by h.p.l.c. as described in the Materials and methods section; (a) control; (b) 2-propyl-2-isopropylacetamide, 0.14 mM; (c) ethanol, 200 mM; (d) isopentanol, 10 mM. The numbers below the bars refer to the numbers of carboxy groups. Each value represents the mean for duplicate dishes with the different individual values indicated by the vertical bar. Total porphyrin levels for all eight dishes: 1.67 ± 0.18 nmol/dish.

vinolate (150 μ M), control cells accumulated mainly protoporphyrin, whereas 2-propyl-2-isopropylacetamide-pretreated cells accumulated 8- and 7-carboxyporphyrin as well (Fig. 1), findings in agreement with those of Marks *et al.* (1983). Ethanol and isopentanol also caused an increased accumulation of 8- and 7-carboxyporphyrin compared with control cells. With ethanol, the accumulation of 8-, 7-, 6- and 5-carboxyporphyrin was dose-dependent and coincided with an equivalent decrease in protoporphyrin (Fig. 2). Piperonyl butoxide, an inhibitor of several different

Fig. 2. Effect of ethanol on porphyrin accumulation from exogenous 5-aminolaevulinate

Cells were plated on 6 cm dishes and were either untreated or exposed to the concentrations of ethanol as indicated for 22 h. Ethanol-treated cells were wrapped in Parafilm and Reynolds 912 film, as described previously (Sinclair *et al.*, 1981) to minimize evaporation. At the end of 22 h, medium was removed to leave a residual volume of 1.5 ml, 5-aminolaevulinate was added to a final concentration of 150 μ M, dishes were rewrapped and incubated for 6 h at 37° in CO₂/air (1:19). At the end of 6 h, dishes were chilled on ice, cells and media harvested together and analysed for porphyrins by h.p.l.c. as described in the Materials and methods section. Each symbol represents the mean of duplicate dishes, with the individual value falling within the symbol unless indicated otherwise. Δ , Total porphyrin; $\bullet$, protoporphyrin; $\circ$, sum of 8- to 5-carboxy-porphyrins.

isoenzymes of cytochrome P-450 (see P. Sinclair *et al.*, 1984a), decreased uroporphyrin accumulation in control cells and prevented the increase caused by ethanol, isopentanol and 2-propyl-2-isopropylacetamide (Table 2).

Correlation between inductions of 5-aminolaevulinate synthase and cytochrome P-450

We compared the effect of increasing concentrations of isopentanol on cytochrome P-450 and 5-aminolaevulinate synthase activity (Fig. 3). We compared the induction of 5-aminolaevulinate synthase by increasing concentrations of isopentanol in the absence and presence of TCB, a chemical shown to cause accumulation of uroporphyrin, with an accompanying decrease in haem synthesis (P. Sinclair *et al.*, 1984b). The enzyme activities are expressed relative to appropriate control values owing to the difference in 5-aminolaevulinate synthase activities in the presence or absence of TCB (see Fig. 3). As shown in Fig. 3, identical relative increases in 5-aminolaevulinate synthase were observed in the presence or absence of TCB. Most interestingly, the extent of increase in

Fig. 3. Relationship between induction of 5-aminolaevulinate synthase and cytochrome *P*-450 by isopentanol

Cells were plated in 10 cm-diameter dishes at approx. 1 mg of cell protein/dish and exposed to isopentanol as indicated. 5-Aminolaevulinate synthase and cytochrome *P*-450 were measured on separate duplicate dishes in the same experiment. (i) 5-Aminolaevulinate synthase: cells were exposed to isopentanol in the absence (a) or presence (b) of 0.86 μ M-TCB. The TCB was dissolved in dimethyl sulphoxide (final solvent concn. 2 μ l/ml of medium). After 20 h, cells were harvested and 5-aminolaevulinate synthase activity measured in sonicated cells as described in the Materials and methods section. Control values (nmol of 5-aminolaevulinate/30 min per mg of protein): (a) 0.16 (0.13, 0.19); (b) 0.85 (0.82, 0.88). (ii) Cytochrome *P*-450: Cells were exposed to isopentanol in the absence of TCB for 22 h. Cytochrome *P*-450 was measured in the 8700 *g* supernatant prepared from cell homogenates as described previously (J. Sinclair *et al.*, 1979). Control levels (nmol of cytochrome *P*-450/mg of 8700 *g* supernatant protein): (a) 51 (48, 54); (b) 37 (37, 37). For ease of comparison, all data were expressed as fold increases over appropriate control values. Each symbol represents the mean for duplicate dishes, with the individual value falling within the symbol unless indicated otherwise. $\circ$, Relative activity of 5-aminolaevulinate synthase; $\bullet$, relative amounts of cytochrome *P*-450.

cytochrome *P*-450 by isopentanol (induced in the absence of TCB) was identical with the increases in 5-aminolaevulinate synthase.

The effects of increasing concentrations of isopropyl alcohol, isobutanol and isopentanol on induction of cytochrome *P*-450 and 5-aminolaevulinate synthase are compared in Fig. 4. Again, a direct correlation was found between the relative increases in both enzymes (correlation coefficient = 0.97).

Fig. 4. Relationship between induction of 5-aminolaevulinate synthase and cytochrome *P*-450 by C₃-C₅ alcohols.

Cells were plated on 10 cm-diameter dishes at approx. 1 mg of cell protein/dish and exposed to increasing concentrations of isopropanol (Δ) or isobutanol ($\blacktriangle$). TCB (0.86 μ M) was added in addition to the alcohols in cells assayed for 5-aminolaevulinate synthase, but was absent in cells treated with the alcohols and assayed for cytochrome *P*-450. 5-Aminolaevulinate synthase and cytochrome *P*-450 were measured on separate duplicate plates in the same experiment by assays described in the Materials and methods section. All data are expressed as fold increases over appropriate control values. $\circ$, Isopentanol data replotted from Fig. 3(a), where TCB was present in cells assayed for 5-aminolaevulinate synthase. $\bullet$, Isopentanol data replotted from Fig. 3(b) where TCB was absent during the treatment with isopentanol.

Time course of 5-aminolaevulinate synthase induction

In previous studies with rats (Shanley *et al.*, 1969; Held, 1977), a single dose of ethanol caused a moderate and transient increase in hepatic 5-aminolaevulinate synthase, with maximal increases in the enzyme occurring at 3 h after an acute dose of ethanol. We measured 5-aminolaevulinate synthase activity at different times of exposure to 200 mM-ethanol, comparing the enzyme activities with those in cells exposed to either isopentanol or 2-propyl-2-isopropylacetamide (Fig. 5). Ethanol caused no consistent increase in 5-aminolaevulinate synthase activity. With isopentanol, induction of this enzyme was maximal at 8–12 h. In contrast, 2-propyl-2-isopropylacetamide caused a continuous increase in 5-aminolaevulinate synthase over the 20 h time course.

Ethanol-mediated repression of induction of 5-aminolaevulinate synthase

The results in Fig 6 demonstrate that ethanol caused a concentration-dependent decrease in the induction of 5-aminolaevulinate synthase by 2-propyl-2-isopropylacetamide and TCB. Isopentanol had no such effects (results not shown). No decrease in activity was observed if ethanol was added directly to the assay or was present

Fig. 5. Time course of 5-aminolaevulinate synthase induction

Cells in 10 cm dishes were either untreated (●) or exposed to ethanol (200 mM, ○), isopentanol (10 mM, ▲) or 2-propyl-2-isopropylacetamide (0.14 mM, △). At each time point, separate plates were harvested and 5-aminolaevulinate synthase measured in cell homogenates (Sinclair & Granick, 1977). Each symbol represents the mean for duplicate samples, with the individual values falling within the symbol unless indicated otherwise.

only during the last 3 h of the 20 h exposure to the inducers. Protein synthesis was either unaffected, or slightly increased, by the ethanol exposure, indicating no general toxicity.

DISCUSSION

We have previously shown that C_2 – C_5 alcohols cause a phenobarbital-like induction of cytochrome *P*-450 in cultured chick-embryo hepatocytes (J. Sinclair *et al.*, 1982a, 1984). A quantitative analysis of structure–activity relationships demonstrated a linear correlation between induction of cytochrome *P*-450 and hydrophobicity of the alcohols (Sinclair *et al.*, 1986). Here we have shown that C_3 – C_5 alcohols also induced 5-aminolaevulinate synthase in cultured chick-embryo hepatocytes (Table 1). A close correlation was found between induction of 5-aminolaevulinate synthase and cytochrome *P*-450 (Fig. 4), suggesting a common mechanism is involved. By inference, induction of 5-aminolaevulinate synthase can also be correlated with the hydrophobicity of the inducing chemicals. A role for hydrophobicity has been suggested for other inducers of 5-aminolaevulinate synthase in the cultured-chick-embryo-hepatocyte system (De Matteis, 1978; Marks, 1978).

As with other inducers of 5-aminolaevulinate synthase (De Matteis, 1978), synergistic induction of this enzyme

Fig. 6. Effect of ethanol on protein synthesis and induction of 4-aminolaevulinate synthase in cells exposed to 2-propyl-2-isopropylacetamide and TCB

Cells plated in 10 cm dishes (approx. 1 mg of cell protein/dish) were exposed to 2-propyl-2-isopropylacetamide (0.14 mM) and TCB (0.86 μ M) for 20 h. Cells on separate dishes were also exposed to increasing concentrations of ethanol for the same time period. Separate sets of duplicate dishes were analysed for 5-aminolaevulinate synthase activity and protein synthesis as described in the Materials and methods section. Each value represents the mean for duplicate plates, with the individual values falling within the symbol unless indicated otherwise.

was observed when an inhibitor of haem synthesis, such as TCB, was included in the treatment along with the alcohols (Table 1). Furthermore, even in the presence of TCB, the induction of 5-aminolaevulinate synthase still paralleled that of cytochrome *P*-450 induced in the absence of TCB (Fig. 3). These data suggest that the key component in the induction of 5-aminolaevulinate synthase is the lipophilicity of the inducer, and that feedback repression by haem has a secondary role to modulate the extent of the stimulation caused by the lipophilic inducer.

A single large dose of ethanol has been shown to induce hepatic 5-aminolaevulinate synthase transiently in rats (Shanley *et al.*, 1968; Rubin *et al.*, 1970; Held, 1977) and humans (Shanley *et al.*, 1969) and in cultured rat hepatocytes (Lane & Stewart, 1983). In contrast, ethanol caused little to no induction of 5-aminolaevulinate synthase in primary cultures of chicken-embryo hepatocytes (Fig. 5; Table 1). However, ethanol decreased the induction of 5-aminolaevulinate synthase in cells treated with the combination of 2-propyl-2-isopropylacetamide and TCB (Fig. 6). Thus, with ethanol constantly present in the medium, any induction caused by ethanol alone may have been counterbalanced by its inhibitory effects. These findings contrast with other studies with cultured chick-embryo hepatocytes and rats, in which ethanol enhanced rather than inhibited the induction of 5-aminolaevulinate synthase by another drug, phenobarbital (Held, 1977; Giger & Meyer, 1980). In the studies with rats, ethanol failed to induce 5-aminolaevulinate synthase if the animals were fed during the treatment (Held, 1977). The studies by Giger & Meyer (1980) with cultured chick

hepatocytes utilized medium containing no glucose or pyruvate. Our experiments were carried out with the same medium but containing 11 mM-glucose and 0.28 mM-pyruvate. We suggest that ethanol may repress 5-aminolaevulinate synthase activity by enhancing the recycling of pyruvate to glucose, since ethanol has been found to enhance gluconeogenesis from pyruvate in isolated chick-embryo hepatocytes (Brady *et al.*, 1979; Schultz & Mistry, 1980), most likely by increasing the NADH-mediated reduction of 2,3-diphosphoglycerate. Glucose or a source of glucose has been found to repress 5-aminolaevulinate synthase in humans and rodents (for a review, see Tschudy, 1978) and in cultured chick-embryo hepatocytes (Giger & Meyer, 1980; Schoenfeld *et al.*, 1982).

2-Propyl-2-isopropylacetamide as well as other inducers of 5-aminolaevulinate synthase, have previously been reported to cause accumulation of uroporphyrin in cultured chick-embryo hepatocytes (Marks *et al.*, 1983; de Verneuil *et al.*, 1983; Ferioli *et al.*, 1984). However, even though uroporphyrin accumulates, enough protoporphyrin is evidently made to allow synthesis of haem for induced cytochrome P-450 (Sinclair *et al.*, 1979).

Uroporphyrin accumulation caused by a number of compounds in cultured chick hepatocytes may result from cytochrome P-450-mediated production of activated oxygen species that cause uroporphyrinogen oxidation rather than from direct inhibition of uroporphyrinogen decarboxylase (Ferioli *et al.*, 1984; P. R. Sinclair, unpublished work). Consistent with this theory, ethanol caused uroporphyrin accumulation from an exogenous source of 5-aminolaevulinate at concentrations of ethanol in the range of its binding constant (100 mM) for cytochrome P-450 (Fig. 3) (J. F. Sinclair, L. M. Zaitlin, E. L. Smith, S. K. Howell, P. R. Sinclair, H. L. Bonkovsky & J. Pomeroy, unpublished work), and piperonyl butoxide, an inhibitor of cytochrome P-450, prevented this effect (Table 2, P. Sinclair *et al.*, 1984a). These results may help to explain the well-known clinical association of excess ingestion of alcoholic beverages with the human disease porphyria cutanea tarda, a disorder characterized by excess hepatic production and urinary excretion of 8- and 7-carboxyporphyrins. (For a review, see Bonkovsky, 1982.)

In summary, C₃–C₅ alcohols induced 5-aminolaevulinate synthase and caused uroporphyrin accumulation in cultured chick-embryo hepatocytes. A direct correlation was found between induction of both cytochrome P-450 and 5-aminolaevulinate synthase. These alcohols are the simplest molecules yet reported to induce both responses.

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