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PORPHYROGENIC PROPERTIES OF THE TERPENES CAMPHOR, PINENE, AND THUJONE

(WITH A NOTE ON HISTORIC IMPLICATIONS FOR ABSINTHE AND THE ILLNESS OF VINCENT VAN GOGH)

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Abstract. Camphor, α-pinene (the major component of turpentine), and thujone (a constituent in the liqueur called absinthe) produced an increase in porphyrin production in primary cultures of chick embryo liver cells. In the presence of desferrioxamine (an iron chelator which inhibits heme synthesis and thereby mimics the effect of the block associated with acute porphyria), the terpenes enhanced porphyrin accumulation 5- to 20-fold. They also induced synthesis of the rate-controlling enzyme for the pathway, 5-aminolevulinic acid synthase, which was monitored both spectrophotometrically and immunochemically. These effects are shared by well-known porphyrogenic chemicals such as phenobarbital and glutethimide. Camphor and glutethimide alone led to the accumulation of mostly uro- and heptacarboxylporphyrins, whereas α-pinene and thujone resulted in lesser accumulations of porphyrins which were predominantly copro- and protoporphyrins. In the presence of desferrioxamine, plus any of the three terpenes, the major product that accumulated was protoporphyrin. The present results indicate that the terpenes tested are porphyrogenic and hazardous to patients with underlying defects in hepatic heme synthesis. There are also implications for the illness of Vincent van Gogh and the once popular, but now banned liqueur, called absinthe.

The acute porphyrias are human diseases characterized biochemically by genetically-determined defects in hepatic heme synthesis. Although the defects in heme synthesis are in-born errors of metabolism and are present throughout life, clinical attacks of acute porphyria are rare prior to puberty, occur more often in women during the luteal phase of their menstrual cycles, and may be precipitated or exacerbated by a variety of nutritional and environmental factors. A number of drugs and other xenobiotics fall into the latter category. Most of them are inducers of hepatic cytochrome(s) P450 and of 5-aminolevulinate (ALA‡) synthase, normally the rate-controlling enzyme of hepatic heme synthesis. This latter induction coupled with the underlying distal defect in heme synthesis, accounts for over-production and over-excretion of ALA, porphobilinogen, and porphyrins which are biochemical hallmarks of acute porphyria [for reviews, see Refs. 1–3].

In general, xenobiotics that increase the demand for hepatic heme synthesis (e.g. by inducing formation of the heme-containing cytochrome(s) P450 or by increasing the rate of breakdown of hepatic heme) are porphyrogenic, particularly in subjects with a defect in normal heme synthesis. Well-known examples of such xenobiotics include barbiturates, hydantoins, and carbamazepine [1–5]. The ability of terpenes to exert similar effects has not received much attention, although the terpene eucalyptol was incriminated in precipitating attacks of acute intermittent porphyria and was found to be porphyrogenic in an experimental system [6]. To learn more about the porphyrogenicity of terpenes, we have examined camphor, thujone, and pinene as potential hepatic porphyrogens in primary cultures of chick embryo liver cells, a widely used experimental model of porphyria [4, 5, 7–10].

MATERIALS AND METHODS

Materials

(+)-Camphor (molecular weight = 152) and α-pinene (molecular weight = 136) [each > 99% pure] were obtained from the Aldrich Chemical Co. (Milwaukee, WI). A highly purified sample of thujone (molecular weight = 152) [97% α-(−) and

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‡ Abbreviations: ALA, 5-aminolevulinate; BSA, bovine serum albumin; CYP2H, cytochrome(s) P450 IIH, the forms of cytochrome P450 in chickens that are induced by phenobarbital and similar compounds and that appear homologous to CYP2B of rodents; DMSO, dimethyl sulfoxide; FeNTA, ferric nitrilotriacetate with a molar ratio of 1 iron:2 nitrilotriacetates; KLH, keyhole limpet hemocyanin; PAGE, polyacrylamide gel electrophoresis; phosphate-buffered KCl, 0.134 M potassium chloride/0.05 M potassium phosphate, pH 7.4; PVDF, polyvinylidenedifluoride; and SDS, sodium dodecyl sulfate.

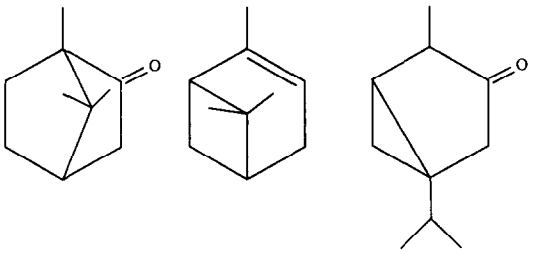

Fig. 1. Chemical structure of camphor, α-pinene, and thujone.

3% β-(+)], supplied in a sealed ampoule, was donated by J. P. Kutney. Chemical structures of the three terpenes tested are shown in Fig. 1. Glutethimide was from the Sigma Chemical Co. (St. Louis, MO). Benzphetamine was a gift from Upjohn Pharmaceuticals (Kalamazoo, MI). Porphyrins and hemin chloride were from Porphyrin Products (Logan, UT). For each experiment, freshly prepared solutions of the above compounds were made in dimethyl sulfoxide (DMSO) (from Sigma) at stock concentrations of 1 M. The volume of DMSO added never exceeded 2 μL/mL culture medium. Solvent controls contained an appropriate volume of DMSO alone. The mesylate of desferrioxamine was obtained from Sigma. Williams E culture medium was prepared from powder (Gibco, Grand Island, NY) with use of water purified by a Milli-Q System (Millipore Corp., Milford, MA). Ferric nitrilotriacetate was prepared as previously described [8]. Other chemicals were of the highest purity available and were obtained from Sigma or Aldrich.

Liver cell cultures

Cultures were prepared [9, 10] from livers of 16- to 18-day-old embryos of Barred Rock chickens obtained from a local supplier (Carousel Farm, Hopkinton, MA). These cultures behave comparably to those prepared from white Leghorn embryos, which were used previously in our laboratory [8–10].

Cultures for porphyrin measurements were prepared in plastic dishes of 3.5 cm diameter with 2 mL medium/dish; those for enzyme assays or protein assays were in plates of 6 cm diameter with 6 mL medium/dish. Eighteen to twenty hours after cells had been plated, the medium was changed to one free of insulin, and suitable amounts of chemicals were added. Concentrations of chemicals given in the paper refer to final concentrations per volume of culture medium. Cells were then returned to the incubator (37°, 95% air, 5% CO_2) for a further 18–20 hr; after which they were harvested, broken by sonication, and used for preparation of cell-free extracts as described previously [8–10].

Assays

Porphyrins. Total porphyrins and approximate proportions of uro-, copro-, and protoporphyrins were estimated fluorimetrically. More detailed separations and analyses were done by HPLC of acid extracts of cells and medium. The procedure used was a modification of a previously published method [11]. Initial experiments with porphyrin standards and cultured cells indicated that extractions with HCl alone, rather than the HCl–acetone mixtures previously used, led to better recoveries of porphyrins, especially those with higher numbers of carboxyl groups. Accordingly, sonicates of cells and medium were routinely extracted with 1 vol. of 0.1 M HCl, and 200 μL of the extract was injected into the HPLC (Waters Baseline 810 System [Waters Division of Millipore, Milford, MA] equipped with a WISP 712 Auto-Sampler and a Shimadzu model RF-551 Fluorescence Detector [Shimadzu Instruments, Gaithersburg, MD]). The stationary phase was a C18 μBondapak Column (Waters). The mobile phase consisted of a gradient of two solvents: solvent A was a 1:1 (v/v) mixture of 0.1 M ammonium phosphate, pH 4.5, and methanol; solvent B was 100% methanol. The gradient went from 70% solvent A and 30% solvent B to 100% solvent B over 7 min at a flow rate of 1.5 mL/min, followed by 100% solvent B for an additional 11 min. Chromatography data were stored, analyzed and displayed with the aid of a NEC Powermate SX Plus computer and model P6200 printer with use of the Baseline version 3.0 Software Package (NEC Technologies, Boxborough, MA).

Enzyme assays. Activities of ALA synthase [10] and heme oxygenase [8–10] were measured spectrophotometrically. Activities of benzphetamine demethylase were measured fluorimetrically on cell sonicates (excitation wavelength = 420 nm; emission wavelength = 514 nm, Ref. 12). The standard assay contained 3 mM benzphetamine, 1.7 mM NADP, 13 mM nicotinamide, 20 mM isocitrate, 9 mM semicarbazide, 12.5 mM magnesium chloride, 0.02 U isocitrate dehydrogenase, and 0.35 mg protein in a final volume of 140 μL. Initial results established that the formation of product was linear for at least 90 min and proportional to the amount of protein up to at least 1.0 mg protein per tube.

Other methods. Antibodies to ALA synthase were prepared as follows: A synthetic oligopeptide corresponding to amino acid residues 607–621 of the deduced sequence of chick liver ALA synthase [13] was synthesized by the microchemical facility of Emory University. This peptide was linked to keyhole limpet hemocyanin (KLH; Sigma) [14], and the resultant product was purified as described [14]. A 0.8-mL portion of an aqueous solution of the peptide–KLH complex containing 200 μg of the synthetic peptide was mixed with 1.6 mL of Freund's complete adjuvant. Ten 0.1-mL portions of the resultant emulsion were injected intradermally at separate sites into each of two female New Zealand white rabbits. Forty-five and ninety days later, booster injections of 200 μg each were given in Freund's incomplete adjuvant. Beginning 30 days after the second booster injection, the rabbits were bled repeatedly. From the sera obtained, IgG and mono-specific, affinity-purified antibodies were prepared according to methods previously described from this laboratory [15].

In initial studies, it was found that the antiserum reacted with two peptides of about 78 and 50 kDa, which likely represent pro-ALA synthase [16] and the major product of limited proteolysis that retains enzyme activity and immunogenicity [16, 17]. Both were present in only small amounts in control cultures but increased markedly following exposure of cultures to glutethimide plus desferrioxamine or succinylacetone.

Western blotting was performed on proteins prepared from sonicated cell culture after sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS/PAGE), as described [15]. Western blots were prepared according to Evans *et al.* [15] with the following modifications. Proteins were transferred overnight onto polyvinylidenedifluoride (PVDF) membranes that had been pre-soaked in 100% methanol for 2 sec and then in water for 5 min. (This and all subsequent steps were carried out at room temperature.) The membranes were prepared and probed for proteins with the Photoblot® chemiluminescent system (Gibco-BRL, Gaithersburg, MD). After the transfer step, membranes were blocked for 3 hr with gentle shaking in the blocking solution provided by the manufacturer and then incubated (3 hr) in anti-ALA synthase IgG diluted to 25 μg/mL in 0.134 M KCl/0.05 M potassium phosphate, pH 7.4, containing 1% bovine serum albumin (BSA) and 0.02% sodium azide (both w/v). The membranes were washed three times (15 min) in phosphate-buffered KCl containing 0.05% (v/v) Tween 20 and then incubated (2 hr) in biotinylated goat anti-rabbit IgG diluted 1:2000 in blocking solution. The membrane was again washed as described above and incubated (1 hr) in a strepavidin-alkaline phosphatase conjugate diluted 1:2500 in blocking buffer. After further washing, the membrane was coated with Lumiphos 530, as recommended by the manufacturer, and exposed to X-ray film (Fuji Photo Film Co., Tokyo, Japan).

Proteins were measured by the bicinchoninic acid method [18] with use of BSA as a standard.

Statistical procedures

Data are presented throughout as means ± SEM (N = 3). Results of typical experiments are presented. Experiments were repeated at least twice, and usually three times, to be certain that the effects observed were reproducible. Data were found to be normally distributed and thus were analyzed by appropriate parametric procedures (*t*-tests or analysis-of-variance). Data sets with significant F statistics in the initial analysis of variance were analyzed further by Neuman–Keuls' and Scheffe's techniques for pair-wise comparisons among treatment groups.

RESULTS

Effects of selected terpenes and other chemicals on accumulation of porphyrins and activity of 5-aminolevulinate synthase in cultures of chick embryo liver cells

Camphor, α-pinene, and thujone produced mild to moderate degrees of porphyrin accumulation in cultures of chick embryo liver cells (Fig. 2). At the highest concentration tested (1 mM), camphor led to the accumulation of 900–1200, α-pinene to 100–150, and thujone to 80–100 pmol total porphyrin/mg protein (control = 19–50). Camphor or glutethimide led to accumulation of predominantly uro- and heptacarboxylporphyrins (Fig. 3, A and B). In contrast, α-pinene or thujone led to the accumulation of predominantly copro- and protoporphyrins (Fig. 3C). Lower concentrations of the terpenes produced lesser degrees of porphyrin accumulation. For example, 100 μM camphor and thujone produced two-thirds less, and 100 μM α-pinene one-third less, than did 1 mM. Concentrations of 1 or 10 μM did not lead to accumulation of porphyrins beyond those observed in cultures exposed only to DMSO (data not shown). At concentrations up to 1 mM none of the terpenes studied appeared toxic to cell cultures since (a) there was no decrease in cell protein content per plate, (b) there was no increase in dead or floating cells, and (c) inductions of ALA synthase and benzphetamine demethylase were exuberant.

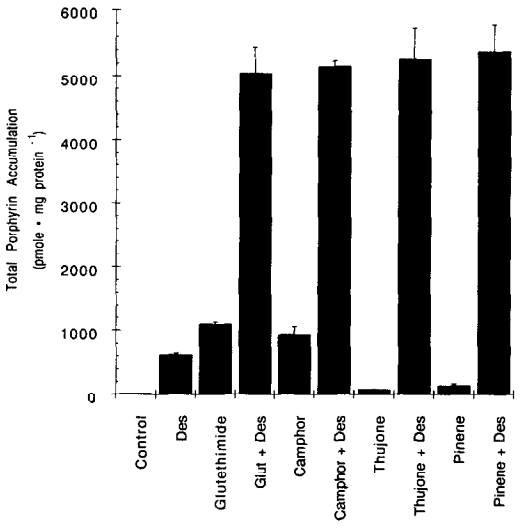

Fig. 2. Effects of selected terpenes, and desferrioxamine (Des) on accumulation of porphyrins in cultures of chick embryo liver cells. Liver cell cultures were prepared on 3.5 cm diameter dishes and harvested as described previously [8]. Porphyrin accumulations were estimated fluorimetrically. Some plates were exposed to terpenes (1 mM), glutethimide (50 μM), or desferrioxamine (Des, 250 μM) for 18–20 hr prior to harvesting. Control plates were exposed to an appropriate volume (2 μL/mL culture medium) of DMSO, the solvent in which the terpenes and glutethimide were dissolved. Results (means ± SEM, N = 3) of a representative experiment are shown. The increases in porphyrin accumulation produced by the terpenes and glutethimide were significantly greater than control ($P < 0.01$); the increases produced by glutethimide were significantly greater ($P < 0.05$) than those produced by α-pinene or thujone, but not different from those produced by camphor.

The iron chelator desferrioxamine enhances the accumulation of porphyrins by blocking conversion

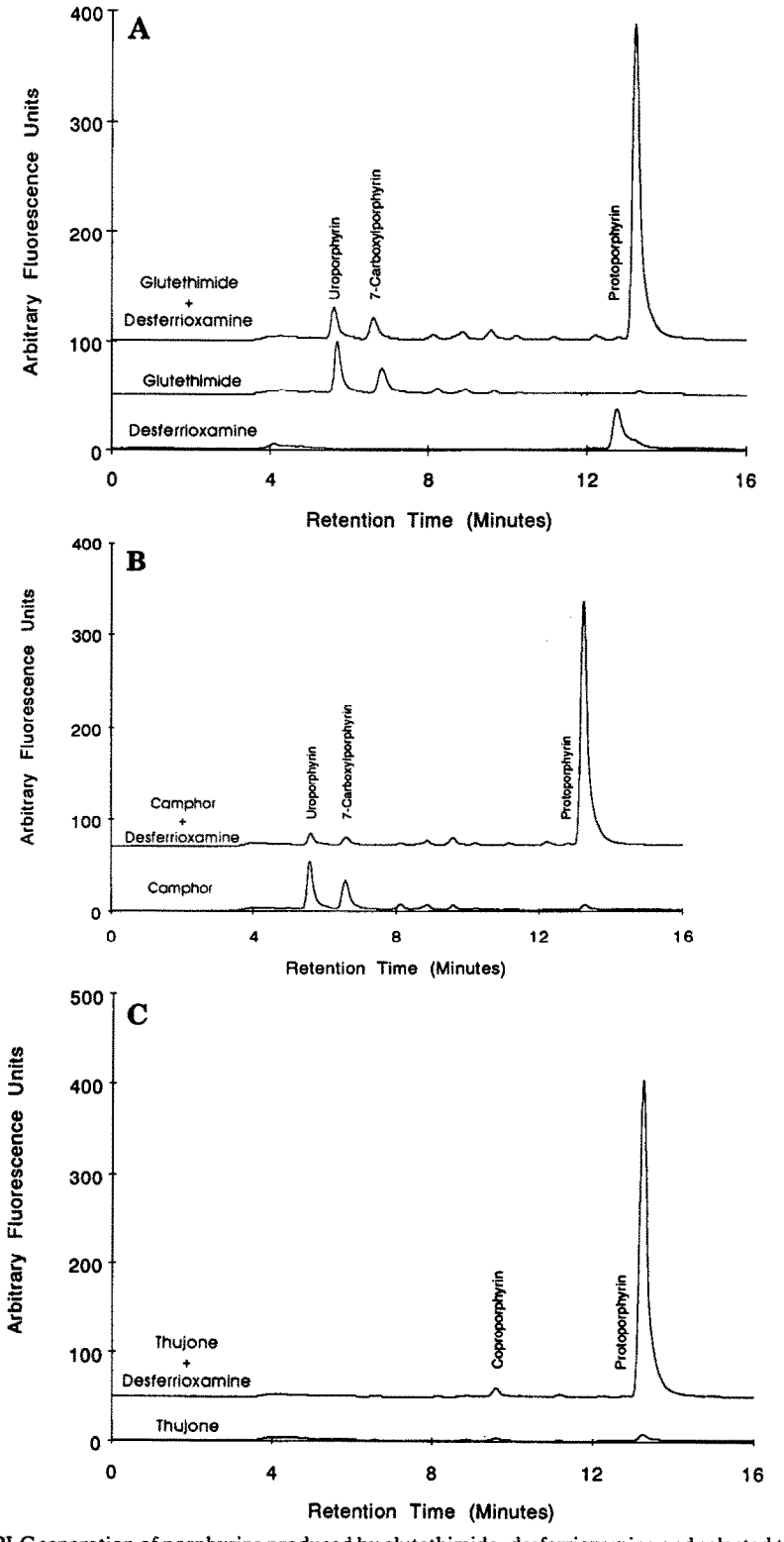

Fig. 3. HPLC separation of porphyrins produced by glutethimide, desferrioxamine and selected terpenes in cultures of chick embryo liver cells. Liver cell cultures were prepared and treated as described in the legend of Fig. 2. The combined cells and medium were extracted with 0.1 M HCl, and 200 μL of the resultant extract was subjected to HPLC with fluorimetric detection as described in Materials and Methods. Panel A shows results for glutethimide (50 μM) with and without desferrioxamine (250 μM); panel B shows results for camphor (1 mM) with and without desferrioxamine (250 μM); panel C shows results for thujone (1 mM), with and without desferrioxamine. (Results for α-pinene were similar.) Desferrioxamine alone produced accumulation of a small amount of protoporphyrin (part A).

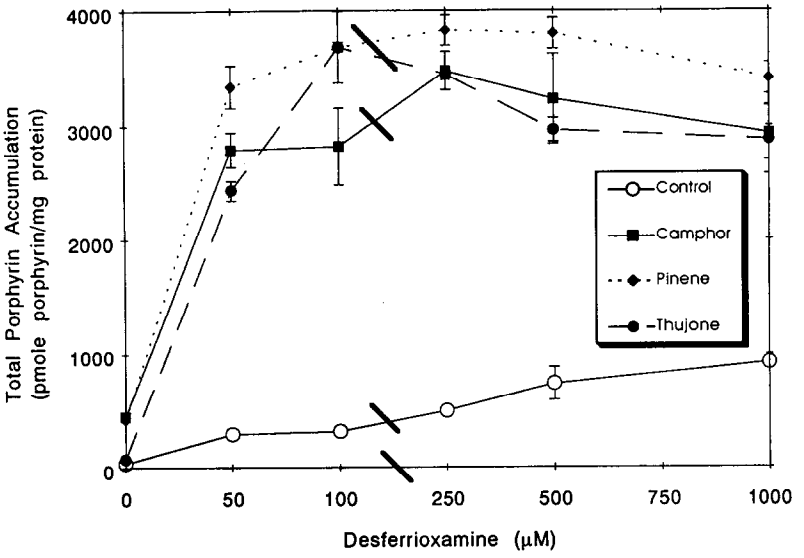

Fig. 4. Effects of increasing concentrations of desferrioxamine on porphyrin accumulation in cultures of chick embryo liver cells also exposed to terpenes. Liver cell cultures were prepared, treated, harvested and analyzed as described in the legend to Fig. 2. Some cultures were also exposed to desferrioxamine (final concentrations as shown), added as the mesylate diluted in water. For each data point, results are means ± SEM, N = 3. Results for glutethimide (50 μM) in the presence of desferrioxamine were virtually identical to those for camphor and, for the sake of clarity, have been omitted.

of protoporphyrin into heme, a step that requires iron [1–3]. The two major causes for protoporphyrin accumulation produced by desferrioxamine are (i) the block in heme formation, and (ii) an increase in the rate of synthesis of protoporphyrin owing to induction of ALA synthase produced by relative heme deficiency. This latter effect is observed most strikingly in the presence of chemicals like glutethimide (Figs. 2–4). Likewise, all the terpenes tested produced a striking synergism in protoporphyrin accumulation in the presence of desferrioxamine (Figs. 2–4), associated with increases in activity (Fig. 5) and amount (Fig. 6) of ALA synthase. However, even in the presence of desferrioxamine, cultures treated with glutethimide of camphor still accumulated small but measurable amounts of uro- and heptacarboxylporphyrins (Fig. 3, A and B).

Effects of selected terpenes and other chemicals on activities of benzphetamine demethylase and heme oxygenase in cultures of chick embryo liver cells

In previous work we showed that glutethimide produces inductions of microsomal cytochrome P450 and heme oxygenase [9, 10], effects confirmed in the current work (Table 1, Fig. 7), in which activity of benzphetamine demethylase was taken as a measure of P450-dependent mixed-function oxidase activity. Camphor was as efficacious as, albeit less potent than, glutethimide as an inducer of benzphetamine demethylase or heme oxygenase. Both the potency and efficacy of α-pinene or thujone were less than those of camphor or glutethimide for induction of benzphetamine demethylase, whereas for induction of heme oxygenase α-pinene was less effective than the other chemicals tested. Addition of desferrioxamine prevented inductions of heme oxygenase by glutethimide and the terpenes (Fig. 7), suggesting that these are heme-dependent events [9, 19]. In contrast, addition of ferric nitrilotriacetate with a molar ratio of 1 iron:2 nitrilotriacetates (FeNTA) (50 μM) with glutethimide or the terpenes produced a synergistic induction of heme oxygenase (results not shown) similar to that previously reported [8, 10].

DISCUSSION

The effects of camphor, α-pinene and thujone on hepatic heme metabolism resemble those produced by glutethimide and other phenobarbital-like drugs. These effects include induction of benzphetamine demethylase (Table 1) and ALA synthase (Figs. 5 and 6). Increased activities of the synthase were associated with increased amounts of its protein (Fig. 6), similar to effects demonstrated for glutethimide (Fig. 6; Ref. 10). Since in untreated liver cell cultures the activity of and protein for ALA synthase are low, and since they increase within a few hours after exposure to glutethimide or terpenes (Figs. 5 and 6; Ref. 10), most, if not all, of the increases in protein and activity of the synthase are probably due to increased rates of message translation. Thus, the increase in activity is probably accounted for principally or totally by an increase in synthesis of the enzyme. Previously, we showed that treatment with glutethimide led to increases in levels of the message for ALA synthase [10].

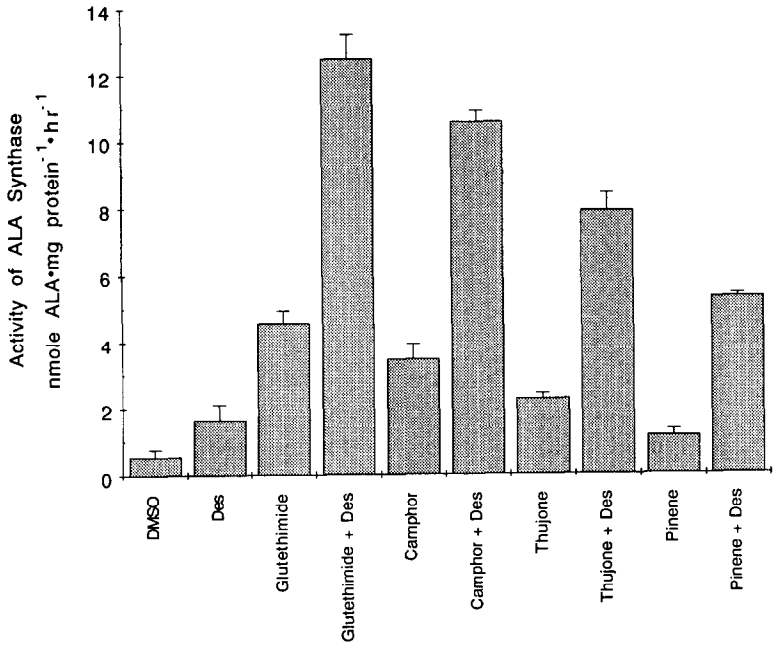

Fig. 5. Effects of glutethimide, selected terpenes, and desferrioxamine (Des) on the activity of 5-aminolevulinate synthase in cultures of chick embryo liver cells. Liver cell cultures on 6 cm plates were prepared and treated as described in the legend of Fig. 2. After 18 hr of treatment, cultures were harvested and the activities of ALA synthase were measured [10]. Results are means ± SEM, N = 3. In the absence of desferrioxamine, the increases produced by glutethimide and camphor were significant ($P < 0.05$). In the presence of desferrioxamine all four chemicals produced significant increases ($P < 0.01$).

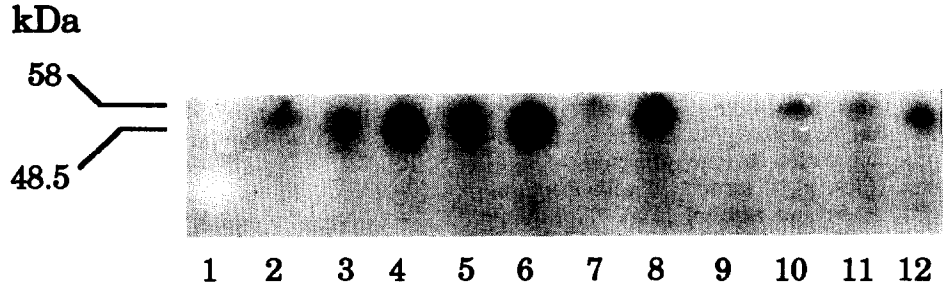

Fig. 6. Western blots of amounts of ALA synthase protein produced in chick embryo liver cells by glutethimide, terpenes, and desferrioxamine. Liver cell cultures were prepared, treated and harvested as described in the legend to Fig. 5. Proteins (1 μg) of cell sonicates were subjected to polyacrylamide gel electrophoresis followed by transfer to PVDF membranes and development with chemiluminescent reagents to detect ALA synthase protein as described in Materials and Methods. The lanes were as follows: (1) molecular mass markers of 58 and 48.5 kDa; (2) DMSO control; (3) 50 μM glutethimide; (4) 50 μM glutethimide plus 250 μM desferrioxamine; (5) 1 mM camphor; (6) camphor plus desferrioxamine; (7) 1 mM thujone; (8) thujone plus desferrioxamine; (9) empty lane; (10) DMSO control; (11) 1 mM pinene; (12) pinene plus desferrioxamine.

Whether the terpenes tested also affect levels or stabilities of the mRNAs for ALA synthase or heme oxygenase is currently under study.

The increase in activity of benzphetamine demethylase produced by the terpenes studied was probably due to a selective increase in cytochrome(s) P450 IIH (CYP2H), induced in chick embryo liver cells by glutethimide and related compounds [8, 9, 20–22]. This form has high activity for demethylation of benzphetamine [12, 23]. Although it has been proposed that induction of cytochrome(s) P450 by glutethimide and similar compounds is a

Table 1. Effects of glutethimide and terpenes on benzphetamine demethylase activity in cultures of chick embryo liver cells

Treatment	Benzphetamine demethylase activity [nmol HCHO · (mg protein)$^{-1}$ · min^{-1}]
Solvent control (DMSO)	0.34 ± 0.02
Glutethimide, 50 μM	1.16 ± 0.01
Camphor, 0.1 mM	1.01 ± 0.05
Camphor, 1 mM	1.29 ± 0.09
α-Pinene, 0.1 mM	0.56 ± 0.02
α-Pinene, 1 mM	0.71 ± 0.01
Thujone, 0.1 mM	0.70 ± 0.05
Thujone, 1 mM	0.94 ± 0.02

Cultures were prepared, treated and harvested, and assays were performed as described in Materials and Methods. Results are means ± SEM for triplicate plates.

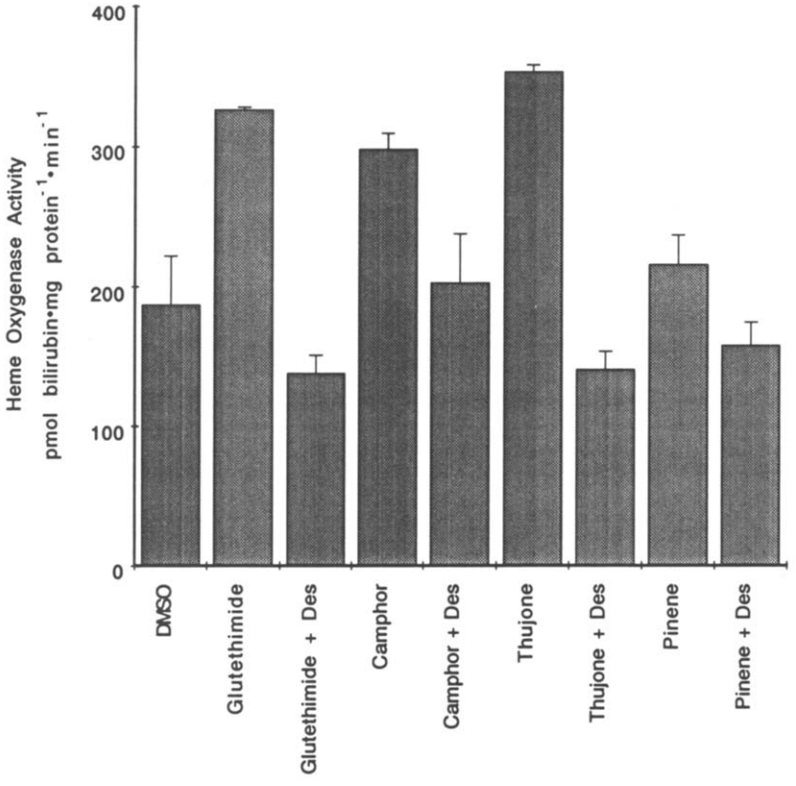

Fig. 7. Effects of glutethimide, terpenes, and desferrioxamine (Des) on the activity of heme oxygenase in cultures of chick embryo liver cells. Liver cell cultures on 6 cm plates were prepared and treated as described in the legend of Fig. 2. After 19 hrs of treatment, cultures were harvested and activities of heme oxygenase were measured [15]. Results are means ± SEM, N = 3. Treatment with glutethimide, camphor or thujone produced significant increases in activity above control ($P < 0.025$), which were prevented by the addition of Des (250 μM). Addition of Des alone had no effect on activity of heme oxygenase (result not shown).

primary event and that induction of ALA synthase follows as a consequence of the relative heme deficiency produced as a result of increased heme utilization for cytochrome P450 [24], the rapidity of the increase in activity and mRNA amounts for ALA synthase after drug treatment makes this interpretation unlikely. Rather, the effects of these drugs on both cytochrome P450 and ALA synthase transcription appear to be virtually simultaneous [22]. Similarly, we recently found evidence for rapid effects of glutethimide on message levels, protein amounts, and activity of heme oxygenase [10, 19].

Whether there is a common mechanism that mediates these rapid drug effects on the two key enzymes of heme metabolism, as well as on CYP2H, remains to be elucidated.

Although the effects of α-pinene and thujone bear similarities to those of glutethimide and camphor (Figs. 2–7, Table 1), they differ in that, in the presence of added iron, they do not produce accumulation of uro- or heptacarboxylporphyrin. Thus, unlike glutethimide, phenobarbital and related compounds, α-pinene and thujone would not be expected to enhance uro- or heptacarboxylporphyrinogen oxidations, perhaps due to a lesser ability to produce oxidizing species in liver cells [8, 25, 26].

Although an experimental model for human acute porphyria in which there is a genetically-determined defect in heme synthesis has not yet been identified, models in which desferrioxamine or other inhibitors of heme synthesis are used have proven useful to elucidate the underlying pathogenesis of disease as well as to provide systems for testing drugs and chemicals as potential porphyrogens. The specific model used in our studies, namely, primary cultures of chick embryo liver cells, has been used widely by us [8–10, 19] and others [4, 5, 7] for this purpose.

The pathogenesis of the neuro-visceral attacks of the acute porphyrias is complex and still not understood completely. However, it involves a partial inherited defect in normal heme synthesis, which, with the addition of other factors, notably exposure to certain drugs or other chemicals, may lead to a marked induction of hepatic ALA synthase with subsequent overproduction of heme precursors. Critical dysfunction of neurons and perhaps muscles is probably a consequence of high levels of ALA and perhaps a low level of heme [27].

The results just summarized establish that the terpenes studied in this work are porphyrogenic and pose a risk to patients with acute porphyria, all of whom have an underlying defect in normal hepatic heme synthesis [1–3].

Vincent van Gogh and terpenes

The postimpressionist artist Vincent van Gogh (1853–1890) executed a large body of paintings and drawings during the last decade of his short and jagged life. His periods of remarkable creativity were interspersed with episodes of debilitating illness. His condition was exacerbated by malnutrition, fasting, overwork, and alcohol abuse, particularly in the form of absinthe. This liqueur was immensely popular in nineteenth century France. In addition to a high concentration of alcohol, it contained a variety of essential oils, and the toxicity of one terpene in particular, thujone, was responsible for its eventual ban [28].

Arnold [29] suggested that van Gogh's fondness for absinthe developed into a proclivity for imbibing terpenes which bordered on a pica; the documented examples were thujone, camphor, and pinene. Camphor was a popular medicament in nineteenth century France; was touted as a panacea by Raspail [30]; and was abused by van Gogh as a home remedy for insomnia [29]. Pinene is the major constituent of oil (essence) of turpentine, which is used for thinning artist's pigments and washing brushes; van Gogh occasionally had to be restrained from drinking turpentine and nibbling his colors [29].

Loftus and Arnold [31] have suggested that Vincent van Gogh suffered from acute intermittent porphyria exacerbated by malnutrition and absinthe abuse. Notwithstanding the acknowledged difficulties of retrospective diagnosis, their unifying hypothesis is the first to successfully address all of the symptomatology, the age of onset (third decade of life), familial illnesses, and the major contribution of the artist's life style toward his medical crises. Historic interest in absinthe, and the illness of Vincent van Gogh, prompted our selection of thujone, pinene, and camphor for this study.

Absinthe contains a number of terpenes and terpene derivatives, including thujone (*ca.* 2.4 mM) and thujyl alcohol (*ca.* 0.8 mM), as well as high concentrations of ethanol (*ca.* 74% by volume) [32]. As usually consumed, absinthe was diluted about 6-fold in water [28]. Thus, the final concentration of these compounds in the drink was about 0.5 mM. Little is known about the pharmacokinetics of these terpenes in humans. If there is an appreciable hepatic first-pass extraction for these compounds, and/or if their rates of hepatic metabolism are not unusually rapid, the concentrations achieved in the livers of absinthe drinkers could easily have been in the 20–200 μM range.* Such concentrations would be sufficient to produce porphyria and induction of hepatic ALA synthase in the presence of a partial block in heme synthesis (Figs. 2–5). An additional effect of ethanol, perhaps acting synergistically, could also be anticipated, since ethanol and other short-chain alcohols found in alcoholic beverages are porphyrogenic, induce ALA synthase and cytochrome P450 [20, 33], and are known to precipitate or exacerbate porphyric attacks [1–3].

In summary, the present results demonstrate that camphor, pinene, and thujone were porphyrogenic in cultured chicken liver cells, and produced inductions of ALA synthase and benzphetamine demethylase. Camphor and thujone also induced heme oxygenase by a heme-dependent mechanism. These compounds pose a threat to patients with acute hepatic porphyrias. The porphyrogenic potential of absinthe (due to thujone and possibly

* The initial hepatic concentration of terpenes is estimated as follows: Assume a normal adult liver weight of 1.5 kg, of which 75%, or 1.125 kg, is water; assume consumption of 500 mL, or about one pint, of diluted absinthe, with a final thujone and thujyl alcohol concentration of 0.5 mM (thus 0.25 mmol imbibed); assume that 50% of these compounds are taken up by "first pass" hepatic extraction of portal venous blood (i.e. an extraction ratio of 0.5). Then, their initial concentration in the liver will be 0.125 mmol/1.125 L or 0.111 mM, equivalent to 111 μM. If, as seems likely for hydrophobic compounds, renal excretion is low, additional amounts of the terpenes would be taken up in subsequent passes through the liver, so that hepatic concentrations might be even higher. Since little is known of the pharmacokinetics of these terpenes in humans, only a rough estimate is possible. However, the above calculation demonstrates that porphyrogenic concentrations of thujone would have been readily achievable in absinthe drinkers.

other terpene constituents), turpentine (pinene), and camphor-containing medicaments is inferred, and may explain some of their deleterious effects on Vincent van Gogh, for whom acute intermittent porphyria has been proposed as the underlying illness.

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