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The site of general anaesthesia and cytochrome P450 oxygenases: similarities defined by straight chain and cyclic alcohols

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1 General anaesthetics disrupt normal cell receptivity and responsiveness while sparing vital respiratory processes. Ultimate elucidation of the molecular basis of general anaesthesia presumes the identification of one or more subcellular components with appropriate sensitivity to the entire array of anaesthetics.

2 Previously, we showed the universal cellular enzymes, cytochrome P450 mono-oxygenases, to be sensitive at relevant concentrations to all anaesthetics tested. The potential significance of P450 inhibition by anaesthetics resides in the contribution of this enzyme family, in conjunction with that of cyclo-oxygenases and lipoxygenases, to the generation from arachidonic acid of lipid second messengers, the eicosanoids.

3 We have shown that P450 enzymes model the site of general anaesthesia in the tadpole with respect to (a) an absolute sensitivity to increasing chain-length series of flexible, straight chain primary and secondary alcohols and straight chain diols, (b) an absolute sensitivity to increasing molecular weight series of rigid cyclic alkanols and cyclic alkanemethanols, (c) the points of abrupt change and of reversal (cut-off) in the linear relationship between increasing anaesthetic potency with increasing carbon chain length, and (d) non-differentiation between secondary alkanol enantiomers. These findings reveal the P450 enzyme family as the most relevant biomolecular counterpart of the site of general anaesthesia, thus far identified.

Keywords: General anaesthesia; anaesthetics; cytochrome P450; eicosanoids; alcohol

Introduction

Identification of a specific biomolecular target appropriately sensitive to the wide array of general anaesthetic compounds has been elusive. In general, the best achievement is the appropriate sensitivity of some functional proteins or protein complexes to some anaesthetics. For example, most enzymes are insensitive to most anaesthetics. Early in this century Harvey showed that bacterial luminescence was depressed by anaesthetics (Harvey, 1915), and in 1965, Ueda found inhibition of firefly bioluminescence by clinical concentrations of certain general anaesthetics (Ueda, 1965). The luciferase-anaesthetic interaction has been examined extensively and that enzyme system championed as a model of the site of general anaesthesia (Curry *et al*., 1990). However, bacterial and firefly luciferases are dissimilar to the site that mediates general anaesthesia by differing up to several fold in concentrations required for half-maximal effects (Curry *et al*., 1990; McKenzie *et al*., 1995), and the enzymes differ from the site of anaesthesia in animals in other ways (Alifimoff *et al*., 1989).

We initiated a systematic study of general anaesthetic action on cytochrome P450 mono-oxygenases upon recognition that these enzymes exhibited an affinity for hydrophobic compounds of diverse chemical structure. We found that eighteen neurodepressant compounds, differing in potencies up to 66,000 fold, at anaesthetic concentrations, inhibited cytochrome P450-mediated metabolism of aminopyrine, an exogenous chemical, and arachidonic acid, an endogenous substrate (LaBella & Queen, 1993). The potential significance of P450 inhibition by anaesthetics lies in the contribution of this universal cellular enzyme family, in conjuction with that of cyclo-oxygenases and lipoxygenases, to the generation of lipid mediators, the eicosanoids, derived from arachidonic acid. We proposed that general anaesthetics perturb the eicosanoid second messenger cascade, and, as a consequence, intracellular and extracellular signalling. Acknowledging, for the wide range of compounds, the impressive correspondence between anaesthetic potencies and enzyme-inhibition potencies, a more detailed study of structure-activity relationships should define more closely the similarities (or differences) between P450 and the site of general anaesthesia. The alcohols have been among the most widely studied compounds in general anaesthesia research, particularly in aquatic species, no doubt because of the compounds' relative non-volatility and miscibility with water, and the availability of different chain lengths, cyclic series and enantiomers. In this paper we describe the effects of alcohols on the NADPH-dependent metabolism of aminopyrine by microsomes prepared from rat liver. Demethylation of aminopyrine appears to be catalysed primarily by P450 isozymes from gene families 2B and 2C (Wolf *et al*., 1990) and 1B and 1C (Ichihara *et al*., 1985). Thus, multiple isozymes are apparently operative in the present experiments.

Methods

Rat liver microsomes

Rat liver microsomes were prepared by the method of Boobis *et al*. (1980). Minced liver tissue (male Sprague-Dawley, 250–300 g) was homogenized with 6–8 passes in a motor-driven Teflon-glass homogenizer with 10 volumes of 0.15 M KCl, 0.25 M potassium phosphate buffer, pH 7.25, containing 1 mM EDTA. The homogenate was centrifuged at $14{,}000 \times g$ for 15 min and the resulting supernatant certrifuged at $200{,}000 \times g$ for 45 min. The microsomal pellet was resuspended in incubation buffer, pH 7.7 at approximately 20 mg protein ml^{-1} and stored at $-80°C$. Total P450 content was determined (Omura & Sato, 1964) to be 0.8–0.9. nmol mg^{-1} protein.

Aminopyrine demethylase

The microsomal suspension in incubation buffer: 50 mM tris pH 7.7, 5 mM $MgCl_2$ and 1 mM EDTF (7.5 ml) was preincubated with or without alcohols in 8 ml capped tubes for 15–20 min at 20°C. Alcohols were added directly, except in the case of the n-

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alkan-1-ol and n-alkan-2-ol compounds $\geqslant$ 6 carbons, cycloalcohols $\geqslant$ 7 carbons and the diols $\geqslant$ 9 carbons; these were added in 5 μl methanol (final concentration of methanol, 15 mM or about 0.03 ED_{50} for anaesthesia), the methanol having no effect on the NASH reaction or enzyme activity. Tubes without substrate and with or without anaesthetic alcohol, served as references. A 940 μl aliquot of suspension was mixed with seven concentrations of aminopyrine (0 and 0.1–2.5 mM) substrate and regenerating system, (glucose-6-phosphate 5 mM, glucose-6-phosphate dehydrogenase 1.0 u ml^{-1}, NADP 0.3 mM) in 1.5 ml capped polypropylene Eppendorf tubes and was incubated at 37°C for 20 min. The reaction was stopped by the addition of 0.3 ml of 20% trichloroacetic acid and the tubes centrifuged at 10 000 *g* for 10 min. The product (formaldehyde) was determined with 0.5 ml supernatant and 0.5 ml NASH reagent (Nash, 1953), incubated for 10 min at 70°C and cooled to room temperature. Absorbance was measured at 412 nm. Typical Michaelis-Menten values were K_m 0.4 mM and V_{max} 4.8 nmol mg^{-1} protein min^{-1}. For each alcohol, the concentration required to inhibit significantly the enzyme activity was determined and a range of concentrations 0.5–2.0 times the K_i was used. In some cases a concentration range of 0.2–2.0 times K_i was used to determine the type of competition. There were no discernible differences in substrate metabolism whether control tubes were capped or uncapped, shaken or unshaken, indicating that oxygen availability was not a limiting factor. The Lineweaver-Burk plot, Michaelis constants (K_m) and (K_i) were determined with the IBM-PC programme ENZYME (obtained from Dr R.A. Lutz, Graben 35, CH-8402 Winterthur, Switzerland), a weighted nonlinear least-squares curve fitting procedure with iterative reweighting (Lutz *et al*., 1986). Weights were set according to an exponential variance model (Munson & Rodbard, 1980). The equation used for the pure competitive mechanism was $K_i = I/K_{(m)}/K_m - 1)$, where $K_{(m)}$ is the apparent K_m in the presence of a specified inhibitor concentration. K_i values are shown as means $\pm$ s.e. ($n = 4-7$).

Difference spectra

Difference spectra (Jefcoate, 1978), were obtained for aminopyrine and alcohols by a Milton Roy Spectronic 3000 Array Spectrophotometer (Milton Roy Company, New Rochelle, N.Y.). A computer software-controlled programme (Rapidscan) plotted the spectral data. The microsomes were diluted in 50 mM potassium phosphate buffer pH 7.0 at 1 mg protein 950 μl^{-1} and a given amount of alcohol dissolved in 5 μl methanol (which did not effect the spectrum) was added 10–15 min before photometer readings. For the LIGAND programme (Munson & Rodbard, 1980), the trough to peak differences (delta A) served as 'amount bound'. 'Nonspecific binding', a term applicable only to radioligand assays, was set to 0. 'Specific activity' was given a value of 1 d.p.m. $pmol^{-1}$ and the concentration of ligand entered as nmol ml^{-1} in the 'Hot only' experimental type of radioligand assay. Multiplication of the calculated K_d by 10^3 was required to give K_d values within 0.5% of theoretical values.

Determination of alcohol concentrations in the enzyme-assay incubation medium

We expolored the possibility that free soluble alcohol was lost through partitioning into the microsomal membranes. After a usual incubation period, tubes were centrifuged and the concentration of alcohol in the supernatant estimated from the absorbance difference-spectrum obtained with 1 mg microsomal protein. Supernatant (no alcohol), plus microsomes served as reference and microsomal suspension plus alcohol as control in the photometric determination.

Materials

Commercial sources of alcohols: methanol, butanol: Mallinkrodt Specialty Chemicals Co.; propanol octanol: Fisher Scientific; ethanol: Manitoba Liquor Commission; pentanol, hexanol, heptanol, nonanol, undecanol: Sigma Chemical Co.; decanol: Eastman Kodak Co.; dodecanol, cycloalkanemethanols, cycloalcohols, *R*-(−) and *S*-(+) enantiomers of 2-pentanol, 2-hexanol, 2-heptanol, 2-octanol: Aldrich Chemical Co.; *R*-(−)-2-butanol and S-(+)-2-butanol: Fluka Chemika-Biochemika. The specified purity for the alcohols as a whole was 97–99% (except for ethanol, 100%; 1,5-pentanediol and cycloheptanemethanol, 96%; 1,7-heptanediol and cyclododecanemethanol, 95%). The specified enantiomeric purity of the *R*(−) and S(+) alcohols was 97–99% (except for *R*-(−)-2 butanol, 89%; *S*-(+)-2 butanol, 92%, and *R*-(−)-2-octanol, 95%). Reagents for the NADPH regenerating system and the NASH reaction were obtained from Sigma-Aldrich Canada Ltd., Mississauga, Ontario and reagents to prepare buffers from Mallinckrodt Chemical (Anachemia Canada Inc., Montreal, Quebec).

Table 1 Concentrations of alcohols needed to inhibit cytochrome P450 activity (aminopyrine demethylase) *in vitro* and to effect general anaesthesia in the tadpole

Alcohol	*No. of carbons*	K_i (μM)	EC_{50}* (μM)
n-Alkan-1-ol	1+	–	590,000 ± 41,000
	2	160,000 ± 17,700	190,000 ± 16,000
	3	29,400 ± 3,900	73,000 ± 2,4000
	4	6,700 ± 170	10,800 ± 770
	5	3,900 ± 390	2,900 ± 110
	6	1,300 ± 190	570 ± 37
	7	230 ± 20	230 ± 11
	8	75 ± 6	57 ± 2.5
	9	30 ± 4	37 ± 2.4
	10	18 ± 2	12.6 ± 0.48
	11	13 ± 2	8.1 ± 0.81
	12	35 ± 4	4.7 ± 0.33
	13	2,660 ± 320	Not anaesthetic
	14	5,490 ± 1,930	Not anaesthetic
n-Alkan-α,ω-diol	5	56,000 ± 5,800	19,000 ± 2,000
	6	13,000 ± 2,450	25,000 ± 5,000
	7	2,880 ± 560	3,300 ± 300
	8	1,330 ± 240	960 ± 90
	9	712 ± 11	640 ± 11
	10	414 ± 11	250 ± 50
	12	64 ± 2	45 ± 15
n-alkan-2-ol‡	4	10,900 ± 570	17,100 ± 110
	5	3,800 ± 150	4,800 ± 270
	6	1,500 ± 130	1,420 ± 79
	7	390 ± 30	330 ± 20
	8	110 ± 10	61 ± 3
Cycloalkanol	5	7,870 ± 680	no data
	6	4,040 ± 90	4,980 ± 470
	7	1,890 ± 160	1,290 ± 170
	8	760 ± 70	390 ± 30
Cycloalkane-methanol	4	25,400 ± 2,900	54,000 ± 3,200
	5	5,520 ± 940	8,400 ± 640
	6	2,680 ± 430	2,700 ± 190
	7	1,220 ± 130	910 ± 17
	8	280 ± 50	320 ± 14
	13	37 ± 10	13 ± 2

For K_i values the means ± s.e.mean are shown. Three to six determinations were made for each alcohol. *EC_{50}: concentration for general anaesthesia (reversible loss of righting reflex) in 50% of the animals (tadpoles). Data for n-alkan-1-ols from (Alifimoff *et al*., 1989), n-alkan-2-ols from (Alifimoff *et al*., 1987), cycloalkanemethanols from (Raines *et al*., 1993), cycloalkanols from (Curry *et al*., 1991) and n-alkan-α,ω-diols from (Moss *et al*., 1991) (with permission).
+ Methanol inerferes with the colorimetric assay.

Results

In Table 1, for five series of alcohols the K_i values for inhibition of aminopyrine metabolism were compared to the concentrations obtained by Miller and co-workers and Franks and Lieb and coworkers to effect anaesthesia in tadpoles (Alifimoff *et al*., 1987; 1989; Raines *et al*., 1993; Curry *et al*., 1991; Moss *et al*., 1991). For each series of alcohols concentration-effect curves are presented in Figures 1 – 5. For the primary alcohols there was a log-linear relationship between increasing carbon chain length and increasing potency, both for enzyme inhibition and anaesthesia (Figure 1) (K_1 for the one-carbon alcohol, methanol, could not be obtained because the 590 mM (ED_{50}) level of that alcohol interfered with the colormetric enzyme assay); the abrupt change in slope occurred at a chain length of eight carbons, and the cut-off point, i.e. reversal of slope, occurred at C12 for both enzyme inhibition and anaesthesia (Table 1). A similar concordance between *in vitro* and *in vivo* responses was seen for the series of the n-alkanediols, again with a change in slope at eight carbons (Figure 2). In the cycloalkanemethanol series C13 was the most potent member to effect anaesthesia and to inhibit P450 activity; cut-off would appear to occur at C13 or beyond (Table 1). Unavailability of congeners with more than eight carbon atoms precluded determination of cut-off for the secondary and cycloalkanol series. For each member of each alcohol series, double reciprocal plots define a classic competitive inhibition of enzyme activity; plots are shown for the eight-carbon member of each series (inserts, Figures 1 – 5). Aminopyrine demethylase inhibition, with a ten fold range of concentrations of n-pentan-1-ol or n-octan-1-ol, was analyzed (ENZYME) for each concentration individually, or for all concentrations simultaneously, resulting in a competitive type inhibition. The overall mean K_i values were 3600 ± 320 μM for pentanol and 57 ± 7 μM for octanol. From these data, a variable slope, sigmoid dose-response analysis with a single dose of substrate, aminopyrine, and a range of alcohol concentrations revealed Hill-slope values of 0.95 ± 0.08 and 0.90 ± 0.14 for pentanol and octanol,

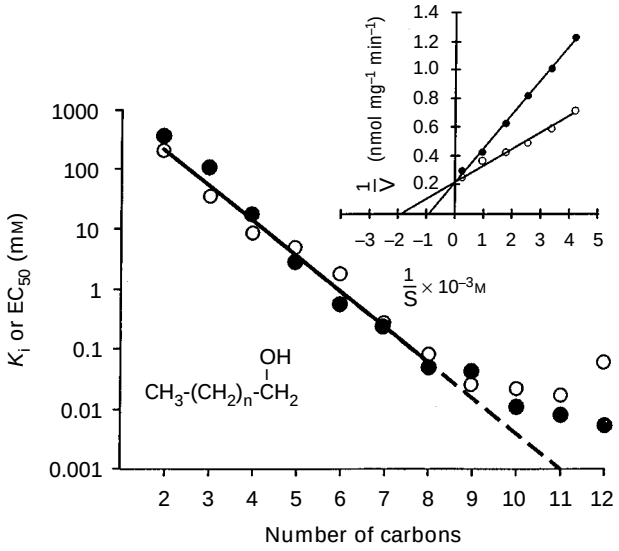

Figure 1 Concentrations of primary alcohols that inhibit P450 *in vitro* (K_i) (○) and effect anaesthesia *in vivo* (EC_{50}) (●) as a function of carbon chain length are shown. The errors are within the size of the symbols. Inset: double reciprocal plot of enzyme activity versus the concentration of the eight-carbon alcohol shows the competitive nature of the inhibition.

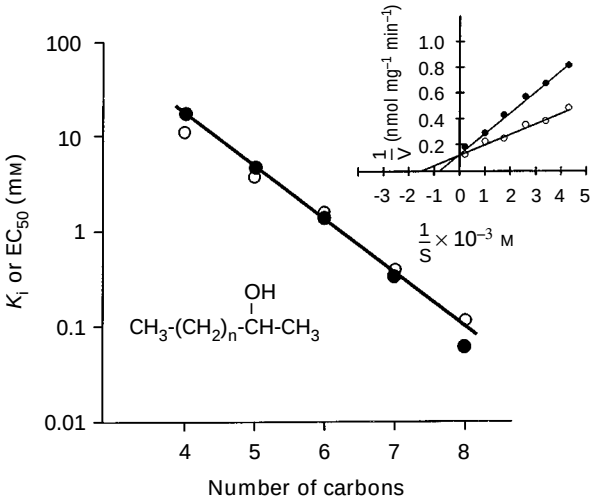

Figure 3 Concentrations of secondary alcohols that (○) inhibit P450 *in vitro* (K_i) and (●) effect anaesthesia *in vivo* (EC_{50}) as a function of carbon chain length. Inset as in Figure 1.

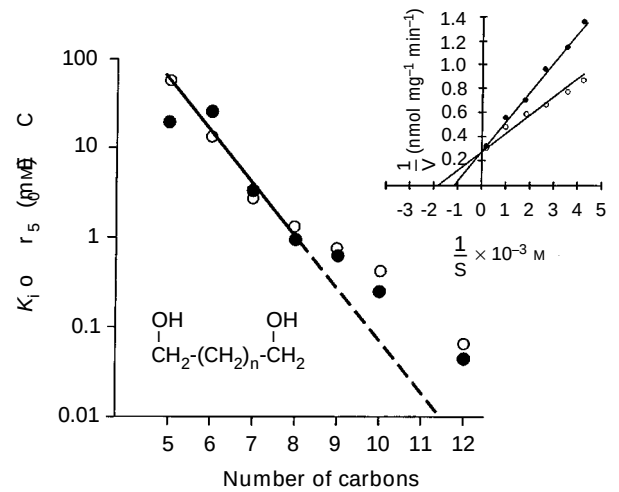

Figure 2 Concentrations of n-alkan-α, ω-diols that (○) inhibit P450 *in vitro* (K_i) and (●) effect anaesthesia *in vivo* (EC_{50}) as a function of carbon chain length. Inset as in Figure 1.

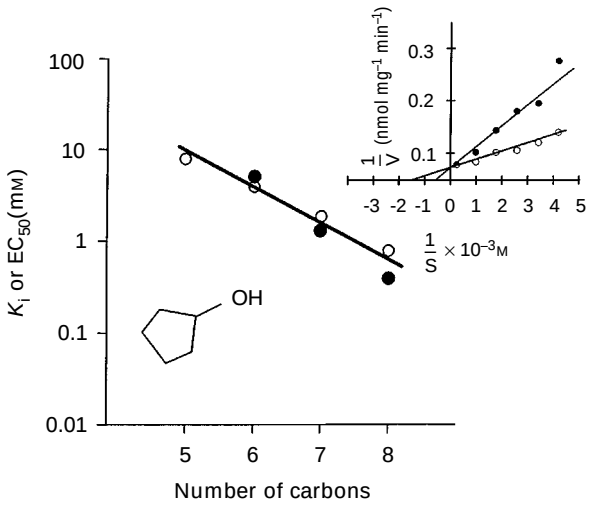

Figure 4 Concentrations of cycloalkanols that (○) inhibit P450 *in vitro* (K_i) and (●) effect anaesthesia *in vivo* (EC_{50}) as a function of carbon chain length. Inset as in Figure 1.

respectively, indicating competitive inhibition. Also indicative of competitive inhibition, the IC_{50} for inhibition of aminopyrine demethylase by cycloheptanol increased to 2.00, 2.25, 2.50, 2.85, 3.65 and 10 mM when tested against corresponding aminopyrine concentrations of 0.238, 0.294, 0.385, 0.556, 1.0 and 5.0 mM. Alcohol levels approximately 3.5 times their respective K_i values inhibited enzyme activity by 90%; higher levels were not tested. No significant differences in potency for inhibition of P450 activity *in vitro* were evident between enantiomer pairs of secondary alcohols (Figure 6), in accordance with the findings obtained for anaesthesia in tadpoles.

The absorbance difference-spectrum obtained with a chemical substance added to a suspension of liver microsomes represents the influence of a given ligand, sequestered within the P450 hydrophobic crypt, on the spin-state of the heme iron atom (Jefcoate, 1978). Virtually all chemical compounds that are inhibitors of or competing substrates for cytochromes P450 yield an absorbance difference-spectrum and exhibit classical competitive inhibition of catalytic activity. In this study each member of each alcohol series generated an absorbance spectrum and was shown to be a competitive inhibitor of P450-mediated aminopyrine demethylase. Most of the alcohols yielded an absorbance spectrum that was very similar to that of the substrate, aminopyrine, indicating a common binding site (Figure 7). Other competitively inhibitory alcohols, i.e. some smaller members, bound less closely to the substrate site (Figure 7a).

The binding affinity of octanol for P450 was determined from spectral data with the LIGAND analysis; a K_s of 47 ± 5 μM obtained in this way is reasonably close to the K_i for enzyme inhibition of 75 ± 6 (Table 1).

Recovery experiments to assess possible loss of alcohol into microsomal membranes indicated, at most, a loss of 15% (Table 2). This value would not seem to alter significantly the K_I/EC_{50} values.

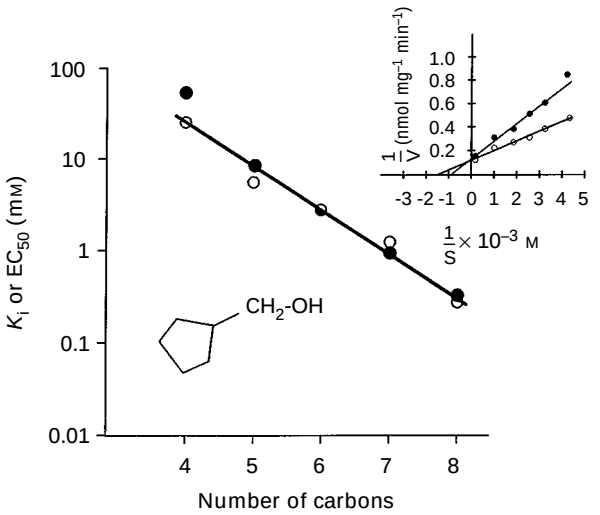

Figure 5 Concentrations of cycloalkanemethanols that (○) inhibit P450 *in vitro* (K_i) and (●) effect anaesthesia *in vivo* (EC_{50}) as a function of number of carbons. Inset as in Figure 1.

Discussion

In this study we showed that P450 enzymes model the site of general anaesthesia in the tadpole with respect to (a) an absolute sensitivity to increasing chain-length series of flexible, straight chain primary and secondary alcohols and straight chain diols, (b) an absolute sensitivity to increasing molecular weight series of rigid cyclic alkanols and cyclic alkanemethanols, (c) the points of abrupt change and of reversal (cut-off) in the linear relationship between increasing anaesthetic potency with increasing carbon chain length, and (d) non-differentiation between secondary alkanol enantiomers. Although the values for both EC_{50} and K_i lie closely around a common line drawn by inspection, fine scrutiny indicates that the apparent geometries of the enzyme pocket (mammalian P450) and anaesthesia site (tadpole) are not identical. There was a trend

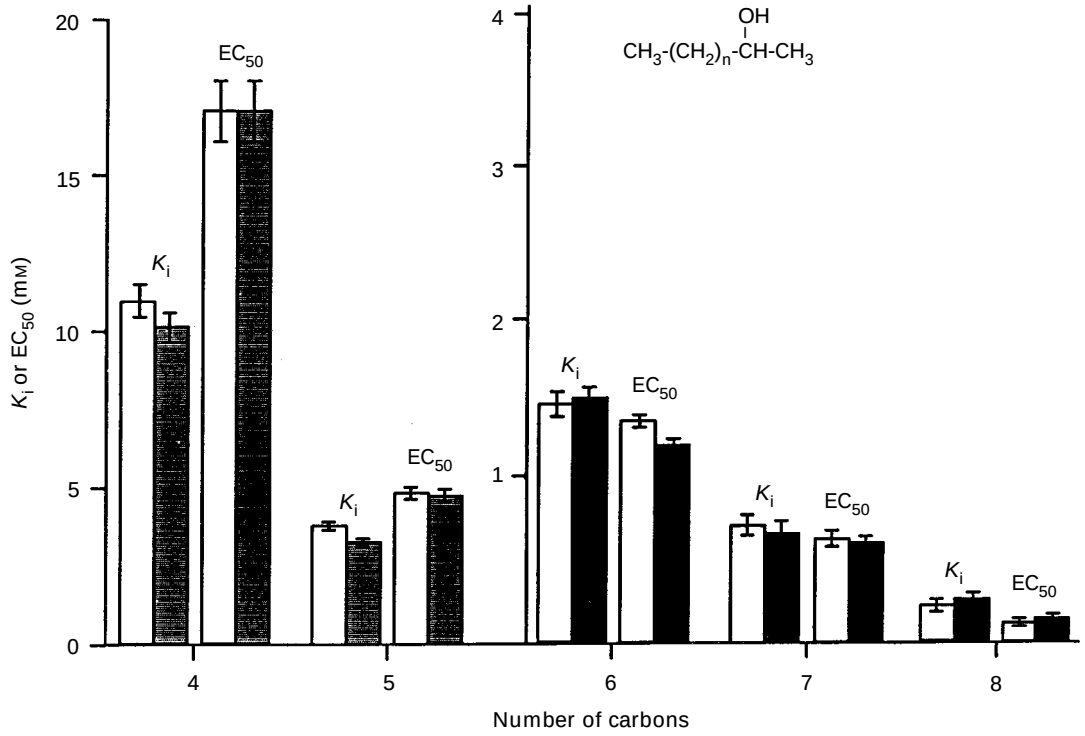

Figure 6 Concentrations of enantiomer pairs of secondary alcohols that inhibit P450 *in vitro* (K_i) and effect anaesthesia *in vivo* (EC_{50}) as a function of carbon chain length. Open columns, **R**(−)-form; shaded/solid columns, **S**(+)-form.

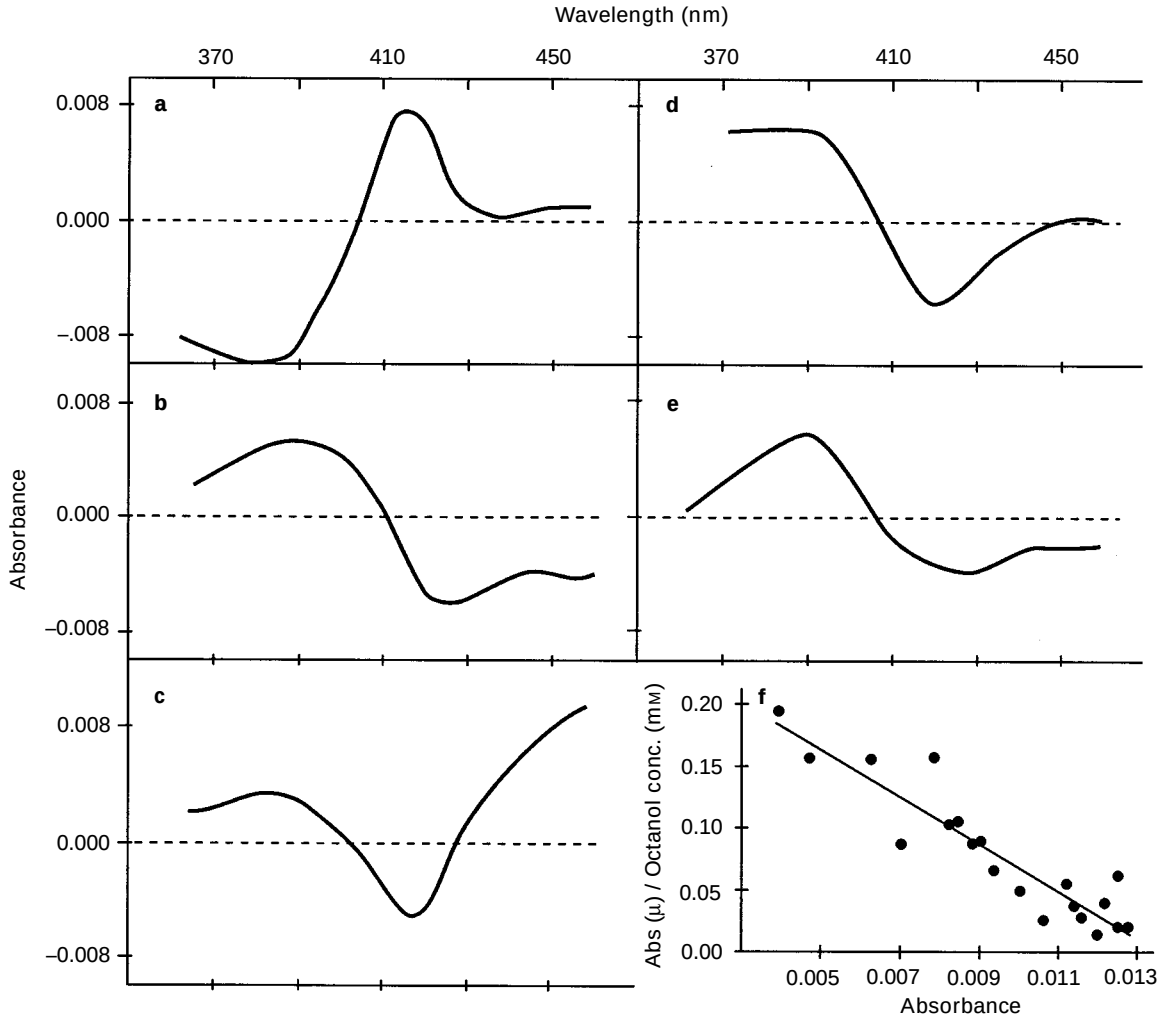

Figure 7 Absorbance difference-spectrum of the alcohol-P450 complex for (a) n-pentan-1-ol, 9 mM; (b) n-octan-1-ol, 0.2 mM; (c) aminopyrine, 6 mM; (d) cyclopentanol, 18 mM; and (e) cyclooctanol, 1 mM (see Methods details). The affinity of octanol for P450 was calculated from spectral data; concentrations of octanol ranged from 20 to 800 μM. The plot shown in (f) yielded a dissociation constant, K_s, of 47 ± 5 μM.

Table 2 Concentrations of alcohols in medium after incubation period

Alcohol	*Conc.* (μM)	*Alcohol recovery* (%)
n-Octan-1-ol	200	112 ± 19
n-Decan-1-ol	30	101 ± 9
Cyclooctanol	500	85 ± 11

See Methods for details

apparent for each of the alcohol series (Figures 1 – 5), whereby the enzyme appeared to be slightly more sensitive than the anaesthesia site to the smaller alcohols and slightly less sensitive to the larger alcohols. The present findings are supported by our earlier study with eighteen anaesthetics, of diverse chemical structure and differing in potency up to 66,000 fold, that inhibited P450-mediated oxidation of synthetic substrates and of arachidonic acid; in that study values for the ratio K_i/EC_{50} varied around unity (LaBella & Queen, 1993).

Several P450 isozymes are involved in aminopyrine metabolism: 1B, 1C, 2B, and 2C. In view of the close correlations we found between K_i and EC_{50} values, none of the isozymes that contribute to animopyrine metabolism must differ significantly from the others in sensitivity to the alcohols. Other data in preliminary communications (see Chen *et al*., 1994a, b) deal with effects of some alcohols on other P450 isozymes. For n-propan-1-ol, n-butan-1-ol, n-heptan-1-ol, and n-butan-2-ol we found K_i values very similar to those obtained here for aminopyrine metabolism with the substrate ethoxyresorufin, shown to be metabolized primarily by 2C6, and with pentoxyresorufin, metabolized by a profile of several isoenzymes, probably distinct from that with aminopyrine as substrate. Arachidonic acid is another substrate that we found to be sensitive to a wide range of anaesthetics including several alkanols (LaBella & Queen, 1993). This natural substrate is metabolized by multiple P450 isozymes 1A2, 2C8, 2C9, and 2E1 in human liver; (Rifkind *et al*., 1995), 4A11 in human kidney (Imaoka *et al*., 1993a), 2E1 in rat liver (Laethem *et al*., 1993), 2C11, 4A2, 2C23, 4A1 in other rat tissues (Imaoka *et al*., 1993b, Nakamura *et al*., 1994; Lin *et al*., 1995; Alkayed *et al*., 1996), 2B4 in guinea-pig lung (Knickle & Bend, 1994) and 2C1, 2C2, 2E1 in rabbit COS-1 cells, liver and kidney (Laethem & Koop, 1992; Laethem *et al*., 1993). Presumably, in rat liver multiple isozymes act upon arachidonic acid. Thus, we suggest that any given concentration general anaesthetics exert a global, or at least a wide ranging, inhibition of P450 isozymes.

The P450-mediated oxidation of aniline is executed primarily by isozymes 2E1 and 1A2 and, to a lesser extent, 1A1, 2B1 and 2C11; aniline binds in the P450 heme cavity in another distinctive mode. This catalytic function is much more sensitive than the activities described above to inhibition by the diverse array of anaesthetics (LaBella & Queen, 1995), and its perturbation probably not relevant to the anaesthetic state.

The light-emitting enzyme luciferase has been proposed as the best model for the site of anaesthesia (Curry *et al*., 1991), but marked differences in sensitivity to anaesthetic agents are found between the enzymes of bacterial or firefly origin and the site of anaesthesia (Curry *et al*., 1990; 1991). As an example, to compare P450s with luciferases, consider the cycloalkanols for which there are data on tadpole anaesthesia for C6, C7 and C8. In our work on P450 enzymes, the K_i/EC_{50} values are 0.81, 1.46, and 1.9, respectively. For firefly luciferase these values are 0.36, 0.43 and 0.36 and for bacterial luciferase 3.9, 8.0 and 13.6 (Curry *et al*., 1991). For the alkane diols our K_i/EC_{50} values for P450 mono-oxygenase activity are similar to those for the cycloalkanols, but the ratios for the luciferases are even more divergent than calculated for the cycloalkanols (Moss *et al*., 1991).

The site of anaesthesia is probably a protein because (a) anaesthetic activity of a substance, although correlated with its lipophilicity, is determined by other molecular specifications including chemical composition, molecular weight, configuration, and stereospecificity, (b) kinetics of the interaction between anaesthetic and functional proteins often indicate competitive antagonism, and (c) the molecular weights of anaesthetic molecules do not exceed those of ligands for identified cell receptors, enzyme active sites and protein hydrophobic pockets in general (LaBella, 1981; Franks & Lieb, 1994). Structure-activity relationships for general anaesthetics in tadpoles led to a conclusion that the site of anaesthesia is a hydrophobic pocket containing water and connected to an aqueous environment by a narrow channel; a binding site volume for the 'anaesthesia site' has been estimated to be about 250 cubic Å (Franks & Lieb, 1987; Curry *et al*., 1991). The crystal structure of cytochrome isozyme BM-3 P450 from *Bacillus megaterium* reveals an active site, heme-cavity accessible through a long hydrophobic channel 8 to 10 Å in diameter, lined with non-aromatic hydrophobic residues, and represents the substrate binding site; at least one molecule of water is contained in the substrate pocket and bound to the iron atom (Ravichandran *et al*., 1993). The drug-binding pocket of mammalian P450 isozyme 1A1 has an access channel about 8 Å in diameter and accommodates molecules whose dimensions range from 250 to 335 cubic Å (Levis *et al*., 1994).

The bulkiness and rigidity of the cyclic alcohols, in contrast to the flexible long-chain alcohols, should restrict the former to fewer anaesthetic target sites. Accordingly, the cycloalcohols, but not the straight chain alcohols, clearly discriminate between bacterial and firefly luciferases (Curry *et al*., 1990; 1991). Thus, it is important to note that both the cyclic and straight chain alcohol series exhibit the same affinities for the P450 mono-oxygenases as for the tadpole site of anaesthesia. Similarly, for the n-alcohols the cut-off for inhibiting firefly luciferase occurs later than that for effecting anaesthesia (Franks & Lieb, 1984), whereas cut-off is the same for P450 inhibition and for anaesthesia. For each alcohol series, insofar as can be ascertained by the availability of congeners, slope changes and slope reversals of carbon number-effect curves are in close accordance for both anaesthesia and P450 inhibition.

If a biomolecule exists that is as sensitive as the organism to the entire array of general anaesthetic agents, then one must consider the possibilities that (a) the biomolecule is perturbed *in situ* at anaesthetic concentrations, and (b) this perturbation may underlie, or at least contribute to, the state of general anaesthesia. Our work was prompted by the increasing awareness in the literature that virtually all general anaesthetic compounds are metabolized by P450 mono-oxygenases. Furthermore, the studies published on P450 enzymes showed K_m or K_i values for general anaesthetics that were usually close to or less than concentrations found to effect general anaesthesia. Thus, a species of biomolecule with a unique affinity for the diverse range of anaesthetic chemical agents appeared to be revealed. Our first publication showed similar K_i and ED_{50} values for a wide variety of chemical structures. In the present paper the similar sensitivities to rigid and cyclic alcohol series and to alcohol enantiomers point, further, to the potential significance of a role for the P450s in general anaesthesia.

In view of the excellent correspondence in physicochemical properties between the site of anaesthesia and cytochromes P450 and the role of those enzymes in the arachidonic acid, second messenger cascade, it seems reasonable to speculate that inhibition by anaesthetics of the mono-oxygenase family, and other lipid oxygenases, i.e. nitric oxide synthase, cyclooxygenase, and lipoxygenase (Chen *et al*., 1994a,b); constitutes a perturbation of an ensemble of second messenger functions that may contribute to, or even underlie, the state of general anaesthesia. In tissues where localization studies on P450 mono-oxygenases have been performed, these cellular enzymes are virtually universal, resident and functional in cellular membranes in general, including mitochondria, plasma membranes nuclear membrane, nucleolar membrane and endoplasmic reticulum (Bresnick *et al*., 1980; Carubelli & McCay, 1989; Ohyama *et al*., 1991; Edwards *et al*., 1991; Loeper *et al*., 1993). In the same vein, the eicosanoids contribute to the mediation of cell responses to virtually every cell stimulus (Smith, 1989). In the nervous system the eicosanoid messengers play a particularly important role in modulation of neuronal excitability and, specifically of global functions such as sleep and wakefulness (Shimizu & Wolfe, 1990). These lipid mediators may act either directly upon channel, receptor and enzyme proteins or indirectly by influencing the activity of other modulating factors. Even if it is assumed that pervasive inhibition of lipid oxygenases constitutes a major correlate of the state of anaesthesia, other functional proteins, e.g. ion channels, are highly sensitive, also, to anaesthetic concentrations of certain agents, and their perturbation would contribute to the overall agent-specific pharmacological profile of a given anaesthetic agent. Notwithstanding this sensitivity, the cut-off point for effects of alcohols on at least some channels differs markedly from the cut-off for anaesthesia (Li *et al*., 1994). Critical evidence that perturbation of enzymes that generate second messengers contributes to the induction of general anaesthesia must derive ultimately from other sources, including examination of profiles of eicosanoids and other mediators in tissues of anaesthetized animals.

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