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INDUCTION OF CYTOCHROME P-450 BY ALCOHOLS AND 4-SUBSTITUTED PYRAZOLES

COMPARISON OF STRUCTURE–ACTIVITY RELATIONSHIPS

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Abstract—A comparison was made between 4-substituted pyrazoles and short-chain alcohols as inducers of cytochrome P-450. A quantitative structure–activity analysis of the data led to the following equations:

(I) Pyrazoles: Log $1/C = 0.85\ (\pm 0.21)$ Log $P + 1.93\ (\pm 0.38)$, $r = 0.970$
(II) Alcohols: Log $1/C = 0.78\ (\pm 0.14)$ Log $P + 1.46\ (\pm 0.13)$, $r = 0.988$

where C is the concentration that caused a 50% increase in cytochrome P-450, P is the partition coefficient between octanol and water, and r is the correlation coefficient. The results suggest that induction of cytochrome P-450 by these compounds depends on hydrophobicity alone. Electronic and steric factors have insignificant roles.

Hepatic cytochrome P-450, which is induced by many xenobiotics [1, 2], consists of multiple forms. This multiplicity is detectable by substrate specificity, amino acid composition, NH_2-terminal sequence, and immunoreactivity [1, 3]. On the basis of these criteria, cytochromes P-450 have been classified as either basal or induced forms, and the latter have been subdivided so far into five groups according to the types of inducing chemicals [1], i.e. the barbiturate-, the polycyclic aromatic hydrocarbon-, pregnenolone-16α-carbonitrile-, isosafrole- and ethanol-groups. However, there is overlap in delineation of the forms of P-450 by these criteria [1].

One step in understanding the induction of these proteins is to define the structural requirements for chemicals that act as inducers. The polycyclic aromatic hydrocarbon group of inducers interacts with a cytosolic receptor [4]. A correlation has been found between binding to this receptor and induction of cytochrome P-450 [5]. For the halogenated biphenyls in this group of inducers, binding to the receptor and induction of P-450 depend on three properties: (1) hydrophobicity, (2) electron withdrawing substituents, and (3) hydrogen bonding [5]. In contrast, structural requirements for the other four groups of inducers of cytochrome P-450 have not been established [6], although, with the barbiturates, there is suggestive evidence for a correlation of induction with lipophilicity [6, 7]. An effective means of defining such structural requirements is available in quantitative analysis of structure–activity relationships (QSAR), a method that has been useful in rationalizing the biological activities of many drugs, antibiotics and enzyme substrates or inhibitors [8], including the polycyclic aromatic hydrocarbon group of inducers of P-450 [5]. In the QSAR approach, measured biological activities for a parent molecule and a series of derivatives are subjected to regression analysis to obtain the best-fitting equation relating biological activity to some combination of hydrophobic, electronic and steric factors for substituents placed on the parent molecule. In a recent application of the QSAR method [9], it was possible to define the structural parameters governing the action of 4-substituted pyrazoles as inhibitors of alcohol dehydrogenase both *in vitro* and in intact hepatocytes.

We have demonstrated previously the induction of cytochome P-450 by ethanol [10] and other simple alcohols [11] in primary cultures of chicken embryo hepatocytes. We report here that 4-substituted pyrazoles, likewise, increased cytochrome P-450 in the cultured hepatocytes, and the derived structure–activity relationship was compared to that for simple alcohols as inducers. With both chemical groups, there was a direct correlation between inducing ability and hydrophobicity.

MATERIALS AND METHODS

Preparation and treatment of cultured chick embryo hepatocytes. Primary cultures of chick embryo hepatocytes were prepared in serum-free medium as described previously [11]. Cells were exposed to inducers in medium containing 20 mM HEPES¶ buffer, pH 7.4, 1 μg dexamethasone/ml, and 0.3 μg,

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¶ Abbreviations: HEPES, 4-(2-hydroxyethyl)-1-piperazone-ethanesulfonic acid; BSA, bovine serum albumin; P-450, cytochrome P-450; and QSAR, quantitative structure–activity relationships.

Table 1. Concentrations for 50% increase in hepatocyte cytochrome P-450 and hydrophobic constants for substituents in 4-X-pyrazoles

X	C (mM)	log $1/C$ (moles/liter) Observed	Calculated*	Δ log $1/C$	log P†
—$NHCOCH_3$	12.7	1.90	1.60	0.30	−0.38
—H	20	1.70	2.15	−0.45	0.26
—NO_2	1.5	2.82	2.43	0.39	0.59
—OC_2H_5	3.3	2.48	2.61	0.13	0.80
—CH_3	2.5	2.61	2.75	−0.14	0.96
—I	0.56	3.25	3.38	0.13	1.70
—$(CH_2)_4CH_3$	0.02	4.70	4.46	0.24	2.96
—$(CH_2)_5CH_3$	0.015	4.81	4.88	−0.07	3.46

* Calculated using Eq. I.
† Values obtained as indicated in Ref. 13.

3,3',5'-triiodothyronine/ml. The duration of exposure was 24 hr for the 4-substituted pyrazoles. For experiments with alcohols, cells were exposed to a range of concentrations of each alcohol for 48 hr before being analyzed for cytochrome P-450. In control experiments, the 48-hr exposure was found necessary for maximal induction of P-450 by ethanol [10] but not by the 4- and 5-carbon chain alcohols. Exposure to isobutanol, isopropanol, pentanol and isopentanol was repeated for 24 hr and the dose–response pattern was identical to the 48-hr exposure for induction of P-450. In the case of ethanol, the dose–response pattern was similar at 24 or 48 hr exposure for induction of P-450 [10], but the amount of P-450 was greater at the later time. All plates containing alcohols were wrapped in Parafilm and Reynolds 912 film, a procedure found to minimize evaporation. 4-Substituted pyrazoles were dissolved directly into medium.

Other assays. Cytochrome P-450, 7-ethoxyresorufin-*O*-deethylase, and benzphetamine-*N*-demethylase activities were measured as described previously [10] in either 8700 *g* supernatant fractions (P-450) or cell homogenates (oxidase activities). Protein was measured by the procedure of Lowry *et al.* [12], using BSA as the standard.

Chemicals. Pyrazoles with the following substitutions at the 4-position: nitro-, cyano-, acetamido-, iodo-, O-ethyl-, hexyl-, and pentyl- were prepared as described previously [9]. 4-Methylpyrazole was purchased from the Aldrich Chemical Co., pyrazole from Sigma.

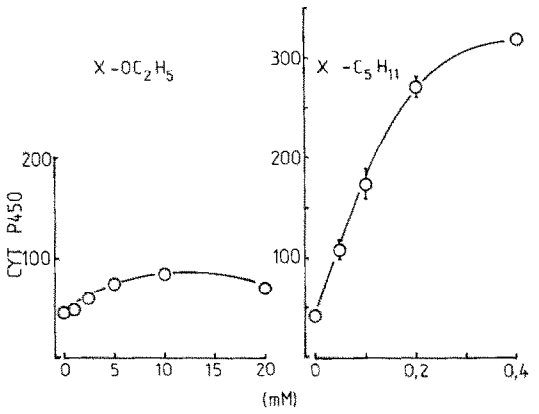
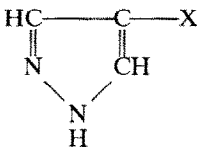


Fig. 1. Induction of cytochrome P-450 by 4-X-pyrazoles. The cells were exposed to the 4-ethoxy- or 4-pentylpyrazoles for 24 hr. Cytochrome P-450 was measured, as indicated in Materials and Methods, and is expressed as pmoles P-450 per mg 8700 *g* supernatant protein. Each value represents the mean of duplicate plates with the individual values falling within the symbols unless indicated otherwise by the bars.

RESULTS

Induction of cytochrome P-450 by 4-substituted pyrazoles or short-chain alcohols



Various concentrations of pyrazole (X = H) and each of seven derivatives, containing the substituents listed in Table 1, were added to hepatocyte cultures to determine the concentration (C) that caused a 50% increase in cytochrome P-450. Figure 1 shows a dose response for 4-pentyl- and 4-ethoxypyrazoles. The values of log $1/C$ for each pyrazole are listed in Table 1. The best-fitting equation relating those values to the chemical nature of the substituents is:

$$\log 1/C = 0.85\ (\pm 0.21) \log P + 1.93\ (\pm 0.38) \quad \text{(I)}$$
$$n = 8,\ s = 0.305\ r = 0.970$$

where n is the number of pyrazoles tested, s is the standard deviation from the regression, and r is the correlation coefficient. P is a hydrophobic parameter for the pyrazole derivative, measured as the partition coefficient between octanol and water [13], and larger values of P indicate molecules that are more lipophilic. The values of P used in deriving Eq. I are indicated in Table 1. In contrast to results obtained with 4-substituted pyrazoles as inhibitors of alcohol dehydrogenase [9], Eq. I indicates that their potency

Table 2. Concentrations for 50% increase in hepatocyte cytochrome P-450 and hydrophobic constants for short-chain alcohols

Alcohol	C (mM)	log $1/C$ (moles/liter) Observed	Calculated*	Δlog $1/C$	log P
Ethanol	50	1.30	1.22	0.08	−0.31
Propanol	28	1.55	1.66	−0.11	0.25
Isopropanol	32	1.49	1.50	0.01	0.05
Butanol	5.5	2.26	2.15	0.11	0.88
Isobutanol	9.5	2.02	2.11	−0.09	0.83
Pentanol	2.3	2.64	2.68	−0.04	1.56
Isopentanol	2.3	2.64	2.57	0.07	1.42

* Calculated using Eq. II.

as inducers of cytochrome P-450 is linearly dependent on hydrophobicity and not influenced significantly by the electronic character of the substituents. In rats and in cultured chick embryo hepatocytes, the deethylation of ethoxyresorufin is a reaction specifically catalyzed by the major MC-inducible isozymes of cytochrome P-450 [11, 14], whereas in rats the demethylation of benzphetamine is greater for the major PB-inducible isozyme of P-450 [14]. When the catalytic activities of the cytochromes P-450 induced by the various pyrazoles were examined, there was no significant increase in ethoxyresorufin deethylase activity and, relative to P-450 concentration, a heterogeneous induction of benzphetamine demethylase activity (results not shown).

Seven short-chain alcohols (Table 2) were also tested to determine the concentration of each that gave a 50% increase in P-450. Using the values for log $1/C$ listed in Table 2, the best-fitting equation is:

$$\log 1/C = 0.78\ (\pm 0.14) \log P + 1.46\ (\pm 0.13) \quad \text{(II)}$$

$$n = 7,\ s = 0.095\ r = 0.988$$

As with the pyrazoles, Eq. II indicates that the potency of these alcohols as inducers of P-450 is linearly dependent on hydrophobicity. Comparing the similar slopes in Eqs. I and II (0.85 vs 0.78) suggests that increasing hydrophobicity by the same amount in a pyrazole substituent and an alcohol will cause similar increases in induction of cytochrome P-450 by these two types of inducers. The larger intercept in Eq. I indicates that, on comparing a pyrazole with an isolipophilic alcohol, the pyrazole would be about three times as potent as an inducer of P-450. However, because of the large standard deviation in Eq. I, more tests would be needed to ascertain the relative effectiveness of these two types of inducers.

DISCUSSION

Our results with 4-substituted pyrazoles and short-chain alcohols show that induction of cytochrome P-450 in cultured chick hepatocytes is, for both sets of compounds, linearly dependent on hydrophobicity. The best-fitting regression equations, with correlation coefficients of 0.970 (pyrazoles, Eq. I) and 0.988 (alcohols, Eq. II), contain only the hydrophobic parameter, P; that is, for the compounds tested in these experiments, induction of P-450 can be rationalized in terms of hydrophobicity alone, and electronic and steric factors have insignificant roles.

In contrast, with the polycyclic aromatic hydrocarbons, steric factors [4] in addition to hydrophobicity, hydrogen bonding, and the electronic character of substituents [5] are significant in the induction of P-450. With the barbiturate group of inducers, there is suggestive evidence that P-450 induction is dependent on lipophilicity [6, 7]. The barbiturates also induce δ-aminolevulinate synthase (ALA-S), the rate-limiting enzyme in heme biosynthesis; there is a direct relationship between the degree of this induction and the lipophilicity of the barbiturate-type of inducer [15, 16].

For the alcohols and pyrazoles, the coefficients of log P were close to 1 (Equations I and II) which might indicate that, in moving to the sites where induction is effected, both the pyrazoles and the alcohols are partitioning between those sites and an aqueous cellular environment in parallel with the way they partition in the reference system, octanol/water. Although this aspect needs to be tested with larger sets of inducers, these results could mean that the inducers are acting at sites that are hydrophobic or that exist in a hydrophobic environment such as the apolar region of membrane lipids. There is recent evidence suggesting that, in phospholipid bilayers and, perhaps, in microsomal membranes, the substrate binding site of cytochrome P-450 is embedded in the apolar lipid region [17]. Bulgheroni [18] recently demonstrated a close correlation between binding to P-450 and induction of the cytochrome by pyridine molecules. Thus, for certain groups of inducers, the structural requirements for inducing and binding to P-450 may be identical.

In cultured chick embryo hepatocytes, all the alcohols presented in this report caused a barbiturate-like response on drug metabolism. In this system, alcohols as well as barbiturates cause pleiotropic increases in (a) the form(s) of cytochrome P-450 having mainly benzphetamine demethylase activity with no increase in ethoxyresorufin deethylase activity [10]; (b) UDP-glucuronyl transferase, utilizing phenol red as a substrate ([10, 11] and unpublished results for 4- and 5-carbon chain alcohols); (c) δ-aminolevulinate synthase [19].

The parent molecule, pyrazole, induces cytochrome P-450 in rats [20] and rabbits [21], and in both animal species the forms induced by pyrazole are similar to those induced by ethanol [21, 22]. Further experiments are necessary to characterize the isozymes of P-450 induced by pyrazoles and alcohols in cultured chick embryo hepatocytes. However, the striking conclusion of the QSAR analysis described here is that alcohols and pyrazoles show the same correlation between induction of P-450 and lipophilicity, with no influence of electronic or steric factors.

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