Changes in the Metabolism of Steroid Hormones by the Rat Liver Cytochrome P-450 System after Induction with *trans*-Stilbene Oxide *

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Drugs, carcinogens, pesticides, and other xenobiotics are well-known substrates for the rat liver microsomal cytochrome P-450 system.[1] However, this enzyme system also has several endogenous substrates, such as steroids, prostaglandins and fatty acids.[2]

It is possible to induce different forms of cytochrome P-450 by administration of different xenobiotics and the best inducers are often poor substrates.[1] The two classical inducers are phenobarbital and 3-methylcholanthrene. The isozymes of cytochrome P-450 have different but overlapping substrate specificities. Thus, phenobarbital preferentially increases the metabolism of substances such as benzphetamine and ethylmorphine,[3,4] while the 3-methylcholanthrene-inducible form, called cytochrome P-448 because of a spectral shift in the absorption maximum of the reduced carbon monoxide complex, preferentially metabolizes substances such as benzo[*a*]pyrene and 2-acetylaminofluorene.[1,5]

We have recently been studying the effects of another type of inducer, *trans*-stilbene oxide, in rat liver.[6] This substance induces the phase II enzymes epoxide hydrolase and glutathione *S*-transferases to a greater extent than do phenobarbital or 3-methylcholanthrene. On the other hand, *trans*-stilbene oxide induces the specific microsomal content of cytochrome P-450 only slightly more than two times.[6,7] It is of interest to investigate whether the forms of cytochrome P-450 induced by *trans*-stilbene oxide are the same as those induced by phenobarbital or 3-methylcholanthrene or are perhaps new isozymes. With the exogenous substrates benzo[*a*]pyrene, ethoxycoumarin, and ethylmorphine the induction by *trans*-stilbene oxide seemed similar to the phenobarbital type[8,9] (unpublished observations in this laboratory).

It is, of course, also important to study the metabolism of endogenous substrates. Steroids are good substrates for studying different forms of cytochrome P-450. The same molecule can be hydroxylated at different positions, while different steroids can be hydroxylated at the same position. Different isozymes of cytochrome P-450 yield characteristic patterns of steroid metabolites. Androgens influence the growth of accessory sex organs and many other tissues.[10] We have used the androgens androstenedione (4-androstene-3,17-dione) and testosterone (17-β-hydroxy-4-androstene-3-one) as substrates to compare the metabolite pattern obtained after administration of *trans*-stilbene oxide, phenobarbital or 3-methylcholanthrene to rats.

Experimental. Male Sprague Dawley rats (Anticimex AB, Sollentuna, Sweden) weighing 180 – 200 g were used in these experiments and given free access to food and water. The rats were injected intraperitoneally with phenobarbital (80 mg/kg body weight in isotonic saline), 3-methylcholanthrene (20 mg/kg in corn oil) or *trans*-stilbene oxide (400 mg/kg in corn oil) once daily for five days. The control animals received the vehicle alone.

The rats were starved overnight before killing by decapitation. The microsomal fraction was prepared from livers in the usual manner[11] and washed once in 0.15 M Tris-Cl, pH 8.0, before resuspension in 0.25 M sucrose. The ^{14}C-labelled steroids were obtained from the Radiochemical Centre, Amersham, England, while the unlabelled steroids were purchased from the Sigma Chemical Co., St. Louis, Mo. All other chemicals used were of analytical grade and were obtained from commercial sources. The measurement of testosterone[12] and androstenedione[13] metabolism was performed with a modified procedure described elsewhere (Meijer, J. and DePierre, J. W., manuscript in preparation). The metabolites of testosterone were separated by thin layer chromatography with the solvent system chloroform – ethylacetate – ethanol (4:1:0.7) and the androstenedione metabolites were separated with chloroform – ethylacetate (4:1).

Table 1. Effects of different inducers on the microsomal content of cytochrome P-450 (nmol/mg protein).[a]

Inducer	Cytochrome P-450
Control	0.37 ± 0.036
Phenobarbital	1.09 ± 0.056
3-Methylcholanthrene	1.41 ± 0.042
trans-Stilbene oxide	0.76 ± 0.071

[a] All assays were performed in duplicate with microsomal fractions prepared from three rats. Values represent means ± SD.

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Table 2. Effects of different inducers on the metabolism of testosterone by the liver microsomal cytochrome P-450 system.[a]

Inducer	Metabolites of testosterone [nmol/min(nmol cytochrome P-450)]			[nmol/min(mg microsomal protein)]		
	6β-OH	7α-OH	16α-OH	6β-OH	7α-OH	16α-OH
Control	2.72±0.46	1.46±0.08	3.64±0.54	0.95±0.10	0.54±0.07	1.28±0.15
Phenobarbital	4.63±0.80	1.17±0.27	3.08±0.42	5.31±1.24	1.35±0.23	3.45±0.44
3-Methylcholanthrene	0.94±0.25	0.71±0.07	0.60±0.18	1.33±0.38	1.00±0.07	0.84±0.22
trans-Stilbene oxide	2.61±0.37	0.80±0.18	3.11±0.29	2.01±0.46	0.61±0.17	2.37±0.34

[a] All assays were performed in duplicate with microsomal fractions prepared from three rats. Values represent means ± S.D.

Table 3. Effects of different inducers on the metabolism of androstenedione by the liver microsomal cytochrome P-450 system.[a]

Inducer	Metabolites of androstenedione [nmol/min(nmol cytochrome P-450)]			[nmol/min(mg microsomal protein)]		
	6β-OH	7α-OH	16α-OH	6β-OH	7α-OH	16α-OH
Control	3.68±0.08	1.65±0.53	5.48±0.41	1.36±0.62	0.62±0.23	2.02±0.17
Phenobarbital	5.20±1.34	1.40±0.16	4.20±0.50	5.72±1.78	1.52±0.14	4.58±0.64
3-Methylcholanthrene	2.27±0.40	1.00±0.11	1.14±0.40	3.19±0.50	1.41±0.11	1.59±0.51
trans-Stilbene oxide	3.90±0.80	1.00±0.09	4.75±0.17	3.02±0.91	0.77±0.13	3.63±0.30

[a] All assays were performed in duplicate with microsomal fractions prepared from three rats. Values represent means ±S.D.

Results and discussion. Phenobarbital, 3-methylcholanthrene, and *trans*-stilbene oxide induced the microsomal content of cytochrome P-450 in these experiments as shown in Table 1.

The formation of different products from testosterone and androstenedione by rat liver microsomes is expressed in Table 2 and 3, respectively. As can be seen, *trans*-stilbene oxide does not change the activities measured in exactly the same manner as either of the other two inducers, but is more similar to phenobarbital than to 3-methylcholanthrene in its pattern of induction. The data are, however, difficult to interprete unambiguously because there are several isozymes involved in the position specific hydroxylation. Both phenobarbital and *trans*-stilbene oxide increase the 16α-hydroxylation of androstenedione and the 6β- and 16α-hydroxylation of testosterone per mg microsomal protein; whereas induction with 3-methylcholanthrene has relatively little effect on these activities. On the other hand, phenobarbital increases the 6β-hydroxylation of androstenedione considerably more than does 3-methylcholanthrene or *trans*-stilbene oxide. In terms of activity per nmol cytochrome P-450 the activity after induction with *trans*-stilbene oxide is rather similar to that after induction with phenobarbital in all cases, but differs dramatically from that seen after induction with 3-methylcholanthrene in the case of 6β- and 16α-hydroxylation of testosterone and 16α-hydroxylation of androstenedione.

In summary, induction with *trans*-stilbene oxide results in several changes in the metabolism of steroids by the cytochrome P-450 system which resemble, but are not identical with the changes observed after induction with phenobarbital. Thus, through their ability to induce the cytochrome P-450 system and thereby alter steroid metabolism, xenobiotics may have profound effects on a large number of physiological functions.

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