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HYPOACTIVITY OF CYTOCHROME P-450 AFTER TRIACETYLOLEANDOMYCIN ADMINISTRATION

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Abstract—In a preceding communication we reported that triacetyloleandomycin (TAO) induces its own transformation into a metabolite forming a stable complex with the iron (II) of reduced cytochrome P-450; the complex is unstable in the ferric state and disrupted by potassium ferricyanide. In this communication, we report the effects of TAO administration on various monooxygenase activities. Repeated administration of TAO, 1 mmole/kg i.p. daily for 4 days, markedly prolonged the hexobarbital sleeping time, decreased the *in vivo* disappearance rate of hexobarbital from the liver, and decreased the *in vitro* activity of hexobarbital hydroxylase, benzo(a)pyrene hydroxylase, ethylmorphine N-demethylase and the *in vitro* conversion of 17β-estradiol into water-soluble metabolites. Hypoactivity of microsomal enzymes was not detected 1 hr after a single dose and was moderate 24 hr after a single dose of TAO, 1 mmole/kg i.p. *In vitro*, addition of 0.3 mM TAO to the incubation mixture decreased hexobarbital hydroxylase activity by only 6% in control microsomes or microsomes from rats killed 1 hr after a single dose of TAO, but decreased it by 30% in microsomes from rats killed 24 hr after a single dose of TAO and by 60% in microsomes from rats killed after repeated doses of TAO. Addition of 50 μM potassium ferricyanide to the microsomes did not modify monooxygenase activities in control microsomes but increased them in microsomes from rats treated with repeated doses of TAO. It is concluded that the administration of TAO decreases several monooxygenase activities and that hypoactivity is at least partly related to the *in vivo* formation, in TAO-induced rats, of an inactive cytochrome P-450–TAO metabolite complex.

In humans, concomitant administration of triacetyloleandomycin (TAO), a macrolide antibiotic, and of ergotamine derivatives, carbamazepine, or oral contraceptives has resulted in ischemic accidents [1], neurologic signs of carbamazepine intoxication [2], and cholestasis of the liver [3], respectively. Administration of TAO has been shown to reduce theophylline clearance [4] and to have a corticosteroid-sparing effect in humans [5]. These various observations suggested that TAO administration may somehow decrease the metabolism of various drugs in humans.

The chemical structure of TAO includes a tertiary amine function, $-N(CH_3)_2$. In a preceding communication [6], we have presented evidence that TAO induces its own demethylation and oxidation into a metabolite which forms a stable complex with the iron(II) of reduced cytochrome P-450. In rats induced by TAO, the complex is formed *in vivo* and can be demonstrated in isolated microsomes. Complexed cytochrome P-450 exhibits a Soret peak at 456 nm, is unable to bind CO, and is unable to demethylate TAO [6]. Treatment of the microsomes with 50 μM potassium ferricyanide oxidizes the iron to the ferric state which is no longer able to bind the TAO metabolite ligand; this regenerates native cytochrome P-450 and restores the CO-binding capacity and the TAO demethylase activity of previously complexed cytochrome P-450 [6]. Whereas previously complexed cytochrome P-450 of TAO-treated rats exhibited a high TAO-demethylase activity, uncomplexed cytochrome P-450 of TAO-treated rats exhibited a poor TAO-demethylase activity [6]. Because dealkylation of tertiary amines is a prerequisite for the formation of complexes [7], it was suggested [6] that induced species of cytochrome P-450 that have a high TAO-demethylase activity are selectively complexed *in vivo* whereas species less active with TAO remain uncomplexed *in vivo* and are barely complexed *in vitro*.

Several compounds transformed into complex-forming metabolites, including piperonyl butoxide and SKF 525-A, are potent inhibitors of cytochrome P-450 activity [7]. In this communication, we report that the administration of TAO markedly reduced several monooxygenase activities.

MATERIALS AND METHODS

Animals, chemicals, and treatments. Animals were male Sprague–Dawley rats, weighing 180–200 g and allowed water and food (Autoclavé 113, UAR, France) *ad lib.* [4-^{14}C]-17 β-estradiol (sp. act., 43 mCi/mmole) was purchased from Commissariat à l'Energie Atomique, Saclay, France; [2-^{14}C]hexobarbital (sp. act., 8.58 mCi/mmole) was pur-

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chased from New England Nuclear, Boston, Massachusetts. TAO, 0.062–1 mmole/kg, was administered i.p. as an acidic solution in HCl (final pH, 2.35); control rats received HCl, pH 2.35; rats were used at different times after a single dose or 24 hr after the last of 4 daily doses.

Drug metabolizing enzymes. The hexobarbital sleeping time was measured as the time interval between loss and recovery of the righting reflex after administration of hexobarbital, 100 mg/kg i.p. The *in vivo* concentrations of unchanged hexobarbital were measured in the liver 10 or 45 min after the administration of 100 mg/kg [2-^{14}C]hexobarbital (sp. act., 25 μCi/mmole) as previously reported [8]. Hepatic microsomes were prepared as previously described [6] and were used immediately thereafter. Unless otherwise indicated, hepatic microsomes were not washed; some microsomes were resuspended in buffer and precipitated three times. The type I binding spectrum of hexobarbital was measured with a microsomal suspension adjusted to 2 mg microsomal protein/ml; after correction of the baseline, hexobarbital, in increasing concentrations, was placed in the test cuvette and the successive binding spectra were recorded on an Aminco DW_2 spectrophotometer. Aniline hydroxylase, ethylmorphine *N*-demethylase, and benzo(a)pyrene hydroxylase activities were measured as previously reported [9]. [2-^{14}C]Hexobarbital hydroxylase activity was measured by the method of Kupfer and Rosenfeld [10]. The transformation of [4-^{14}C]-17 β-estradiol into water soluble metabolites was measured by the method of Jellinck and Perry [11]. Unless otherwise stated, the substrate concentration was 0.5 mM for ethylmorphine, 0.25 mM for hexobarbital, 0.2 mM for benzo(a)pyrene and 0.01 mM for 17 β-estradiol; the kinetics of hexobarbital hydroxylase were measured with various concentrations of hexobarbital. Unless otherwise indicated, enzyme activities were measured in microsomes not treated with potassium ferricyanide; in some tests, 50 μM potassium ferricyanide was added to the microsomes prior to their addition to the incubation mixtures.

In rats killed 1 hr or 24 hr after a single dose of HCl or 24 hr after 4 daily doses of HCl, none of the various activities measured in this study was different from that in control rats.

Antibiotic concentrations, liver histology and ultrastructure. Animals were killed 1 hr or 24 hr after a single dose of TAO, 1 mmole/kg i.p., or 24 hr after the last of 4 daily doses, 1 mmole/kg i.p. daily. The livers were removed and homogenized in 0.1 M sodium potassium phosphate buffer, pH 8. The antibiotic activity in the liver was tested on *Sarcina lutea* [12] and compared to that of liver homogenates from control rats containing various concentrations of oleandomycin base.

Blood was drawn from the inferior vena cava and serum glutamic pyruvic transaminase (SGPT) activity was measured by the method of Reitman and Frankel [13]. Liver fragments were placed in Bouin's fluid, embedded in paraffin 24 hr later, cut and stained with hematoxylin and eosin; other liver fragments were fixed with glutaraldehyde, post-fixed with osmium tetroxide and embedded in epoxy-resin. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a Siemens Elmiskop I A electron microscope.

RESULTS

Effect of TAO administration on monooxygenase activities. 1 hr after a single dose of TAO, 1 mmole/kg i.p., the hexobarbital sleeping time and monooxygenase activities were not different from those in control rats (Table 1). Hexobarbital hydroxylase activity was still unchanged 6 hr after a single dose of TAO, was not significantly decreased 12 hr after the administration of TAO, but was significantly decreased 24 hr after the administration of TAO (Table 1). At that time, ethylmorphine *N*-demethylase activity and benzo(a)pyrene activity were also slightly decreased and the hexobarbital sleeping time was slightly prolonged (Table 1).

24 hr after the last of 4 daily doses of TAO, 1 mmole/kg i.p. daily, the hexobarbital sleeping time was markedly prolonged (Table 1), the disappearance rate of hexobarbital from the liver was markedly reduced (Fig. 1), and monooxygenase activities were severely depressed (Table 1). Three successive washings of the microsomes with buffer failed to improve the reduced hexobarbital hydroxylase activity (not shown). The decrease in the hexobarbital hydroxylase activity was mainly due to a decrease in the apparent V_{max} (Fig. 2); the amplitude of the type I binding spectrum of hexobarbital was not modified at low concentrations of hexobarbital but increased at high concentrations of hexobarbital (Fig. 2).

24 hr after the last of 4 daily doses of TAO, 0.062 or 0.25 mmole/kg i.p. daily, the hexobarbital sleeping time was slightly prolonged and the activity of hexobarbital hydroxylase was slightly reduced but other monooxygenase activities were not significantly decreased (Table 1).

Effect of potassium ferricyanide on monooxygenase activities. Addition of 50 μM potassium ferricyanide to the microsomes prior to their incubation with cofactors and various substrates did not modify monooxygenase activities in microsomes from control rats (Table 2). In microsomes from rats treated with 4 daily doses of TAO, 1 mmole/kg i.p. daily, addition of potassium ferricyanide increased ethylmorphine *N*-demethylase activity by 200 per cent, benzo(a)pyrene hydroxylase activity by 240 per cent, the conversion of 17 β-estradiol into water soluble metabolites by 30 per cent and hexobarbital hydroxylase activity by 50 per cent; however, enzyme activities after addition of potassium ferricyanide remained equal to, or lower than, those in microsomes from control rats (Table 2).

In vitro effect of TAO on hexobarbital hydroxylase activity. Addition of 0.3 mM TAO to the incubation mixture (Table 3) decreased the hexobarbital hydroxylase activity by only 6 per cent in microsomes from control rats and in microsomes from rats killed 1 hr after a single dose of TAO; addition of TAO decreased hexobarbital hydroxylase activity by 30 per cent in microsomes from rats killed 24 hr after the administration of a single dose of TAO, and by 60 per cent in microsomes from rats killed after 4 daily doses of TAO, 1 mmole/kg daily (Table 3). Decreased activities occurred without any detectable

Table 1. Effect of TAO administration on various monooxygenase activities*

	Ethylmorphine *N*-demethylase	Benzo(a)pyrene hydroxylase	Conversion of 17β-estradiol into water-soluble metabolites	Hexobarbital hydroxylase	Hexobarbital sleeping time (min)
	(nmoles/min/mg microsomal protein)				
Control	1.7 ± 0.3	0.23 ± 0.03	0.29 ± 0.10	1.8 ± 0.7	22 ± 8
1 hr after TAO, 1 mmole/kg	1.6 ± 0.7	0.26 ± 0.08	0.25 ± 0.12	1.7 ± 0.7	27 ± 9
24 hr after TAO, 1 mmole/kg	1.4 ± 0.4†	0.10 ± 0.06†	0.26 ± 0.10	1.2 ± 0.7†	40 ± 18†
TAO, 1 mmole/kg daily for 4 days	0.6 ± 0.2†	0.06 ± 0.02†	0.12 ± 0.02†	0.3 ± 0.1†	168 ± 58†
TAO, 0.25 mmole/kg daily for 4 days	1.4 ± 0.5	0.16 ± 0.07	0.29 ± 0.09	0.9 ± 0.4†	58 ± 26†
TAO, 0.062 mmole/kg daily for 4 days	1.4 ± 0.3	0.21 ± 0.06	0.28 ± 0.08	1.3 ± 0.3†	39 ± 18†

* Rats were killed 1 hr or 24 hr after a single dose of TAO, 1 mmole/kg i.p., or 24 hr after the last of 4 daily doses of TAO, 0.062, 0.25, or 1 mmole/kg i.p. daily. Enzyme activities were measured with microsomes not treated with potassium ferricyanide. Results are means ± S.D. for at least 10 rats.
† Significantly different from that in control rats, $P < 0.05$ (*t*-test for independent data).

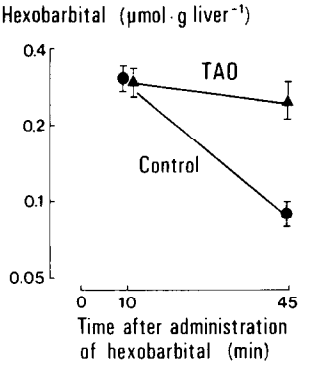

Fig. 1. Effect of TAO administration on the disappearance rate of hexobarbital from the liver. Rats were used 24 hr after the last of 4 daily doses of TAO, 1 mmole/kg i.p. daily. [H-^{14}C] Hexobarbital, 100 mg/kg, was administered i.p. and the concentration of unchanged hexobarbital was measured in the liver 10 or 45 min later. Results are means ± S.E.M. for 4 rats.

decrease in the CO-difference spectrum of the dithionite-reduced incubates (Table 3).

Other studies. Antibiotic activity in the liver was equivalent to that of 0.29 ± 0.09 mM oleandomycin base (mean ± S.E.M. for 5 rats) in rats killed 1 hr after a single dose of TAO, to 0.04 ± 0.01 mM in rats killed 24 hr after a single dose of TAO, and to 0.16 ± 0.01 mM in rats killed 24 hr after the last of 4 daily doses of TAO, 1 mmole/kg daily. SGPT activity in all groups of rats treated with TAO, was not different from that in control rats (not shown). In rats treated with 4 repeated doses of TAO, 1 mmole/kg daily, histologic examination showed no liver necrosis; ultrastructural examination showed dilatation of the endoplasmic reticulum but no ultrastructural sign of necrosis (not shown).

DISCUSSION

Hypoactivity of microsomal enzymes; relationship to induction. Administration of TAO markedly reduced the hexobarbital sleeping time as well as the *in vitro* activity of hepatic microsomes with various substrates (Fig. 1 and Table 1). Decreased activity of microsomal enzymes may offer a possible explanation for several drug interactions previously observed in humans [1–5]. Hypoactivity was not related to hepatic necrosis which was found neither on histological nor ultrastructural examination of the livers. Hypoactivity was not related to destruction of cytochrome P-450 whose total concentrations were in fact increased [6]. Indeed, hypoactivity seemed curiously associated with induction of hepatic drug metabolizing enzymes. Administration of TAO, 1 mmole/kg i.p. daily for 4 days, increased the liver weight/body weight ratio, hepatic microsomal protein concentration, NADPH–cytochrome *c* reductase activity, and total cytochrome P-450 concentration [6]; induction of cytochrome P-450 was progressive, being undetected 1 hr after 1 dose, moderate 24 hr after 1 dose, and marked after 4 daily doses of TAO, 1 mmole/kg i.p. daily; it was dose-dependent being moderate with low repeated doses

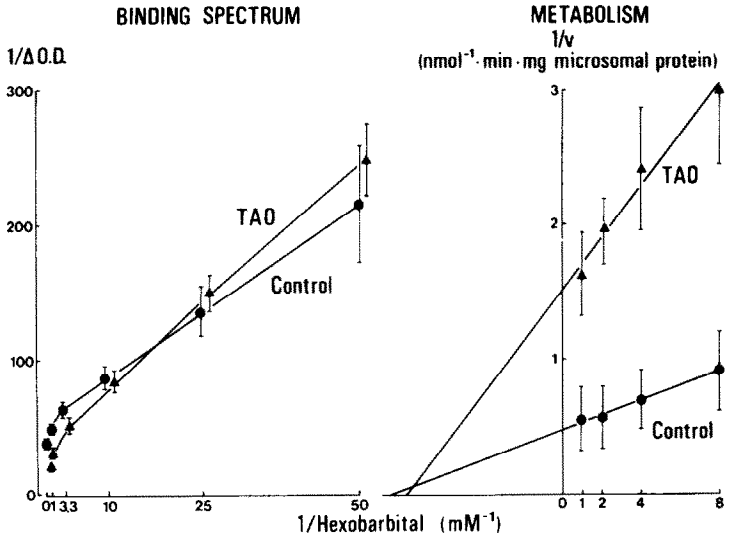

Fig. 2. Effect of TAO administration on the type I binding spectrum and the hydroxylation of hexobarbital. Microsomes were isolated from rats killed 24 hr after the last of 4 daily doses of TAO, 1 mmole/kg i.p. daily; microsomes were not treated with potassium ferricyanide. On the left side of the figure is shown a double reciprocal plot of the amplitude of the type I binding spectrum obtained by adding hexobarbital, in various concentrations, to microsomal suspensions adjusted to 2 mg microsomal protein/ml. Points and bars represent means ± S.E.M. for 7 experiments. The K_s for the linear part of the curve was 0.06 mM hexobarbital in control microsomes and 0.10 mM in microsomes from TAO-treated rats. On the right side of the figure is shown a double reciprocal plot of the amount of hydroxyhexobarbital formed in the presence of various concentrations of hexobarbital. Points and bars represent means ± S.E.M. for 3 experiments. The K_m for hexobarbital was 0.11 mM in microsomes from control rats and 0.12 mM in microsomes from TAO-treated rats; the V_{max} was 2.1 nmoles hydroxyhexobarbital formed/min/mg microsomal protein in control rats but only 0.6 in microsomes from TAO-treated rats.

[6]. Similarly, hypoactivity of microsomal enzymes was not detected 1 hr after 1 dose, was moderate 24 hr after 1 dose and marked after 4 daily doses of TAO, 1 mmole/kg i.p. daily (Table 1); it was dose-dependent, being moderate after low repeated doses of TAO (Table 1). These observations suggest a paradoxical relationship between induction and hypoactivity of microsomal enzymes. TAO induces species of cytochrome P-450 with a high binding capacity for TAO; some of the induced species have a high demethylase activity with TAO and transform TAO into a metabolite which forms a stable complex with hepatic cytochrome P-450 [6]. Under these circumstances, 3 different mechanisms could explain

Table 2. Effect of potassium ferricyanide on various monooxygenase activities*

	Ethylmorphine *N*-demethylase	Benzo(a)pyrene hydroxylase	Conversion of 17 β-estradiol into water-soluble metabolites	Hexobarbital hydroxylase
	(nmoles/min/mg microsomal protein)			
Microsomes from control rats, without potassium ferricyanide	1.8 ± 0.2	0.24 ± 0.04	0.24 ± 0.08	1.7 ± 0.6
Microsomes from control rats, with potassium ferricyanide	1.9 ± 0.5	0.24 ± 0.04	0.22 ± 0.08	1.7 ± 0.4
Microsomes from TAO-treated rats, without potassium ferricyanide	0.7 ± 0.3	0.05 ± 0.02	0.11 ± 0.04	0.2 ± 0.1
Microsomes from TAO-treated rats, with potassium ferricyanide	2.1 ± 0.8†	0.17 ± 0.05†‡	0.14 ± 0.04†	0.3 ± 0.1†‡

*Microsomes were prepared from control rats or from rats killed 24 hr after the last of 4 daily doses of TAO, 1 mmole/kg i.p. daily; 50μM potassium ferricyanide was added (in a few μl of water) to part of the microsomal suspension while the same amount of water was added to the other part. After 5 min, microsomal suspensions were incubated with an NADPH-generating system and with various substrates. Results are means ± S.D. for at least 6 rats.

† Significantly different from that in microsomes from TAO-treated rats, without potassium ferricyanide, $P < 0.05$ (*t*-test for paired data).

‡ Significantly different from that in microsomes from control rats, with potassium ferricyanide, $P < 0.05$ (*t*-test for independent data).

Table 3. *In vitro* effect of TAO on hexobarbital hydroxylase activity*

	Hexobarbital hydroxylase activity		Uncomplexed cytochrome P-450 after incubation	
	without TAO (nmoles/min/mg microsomal protein)	with 0.3 mM TAO (nmoles/min/mg microsomal protein)	without TAO (nmoles/mg microsomal protein)	with 0.3 mM TAO (nmoles/mg microsomal protein)
Control	1.7 ± 0.8	1.6 ± 0.7†	0.6 ± 0.1	0.6 ± 0.2
1 hr after TAO, 1 mmole/kg	1.7 ± 0.7	1.6 ± 0.8†	0.7 ± 0.1	0.7 ± 0.1
24 hr after TAO, 1 mmole/kg	1.1 ± 0.6	0.8 ± 0.4†	0.7 ± 0.1	0.7 ± 0.1
TAO, 1 mmole/kg daily for 4 days	0.2 ± 0.1	0.08 ± 0.03†	0.6 ± 0.1	0.6 ± 0.1

* Rats were killed 1 hr or 24 hr after a single dose of TAO, 1 mmole/kg i.p., or 24 hr after the last of 4 daily doses of TAO, 1 mmole/kg i.p.. Microsomes were incubated at 37° with 0.25 mM [2-^{14}C]hexobarbital and a NADPH-generating system, either in the presence of 0.3 mM TAO added in 25 μl methanol or in the presence of 25 μl methanol. After 15 min of incubation, part of the incubate was immediately used to measure the CO-difference spectrum of the dithionite-reduced incubates; the other part was used to measure formed hydroxyhexobarbital. Results are means ± S.D. for at least 6 rats.

† Different from that without TAO, $P < 0.05$ (*t*-test for paired data).

an induction-mediated hypoactivity of cytochrome P-450: (a) the formation, in induced microsomes, of an inactive cytochrome P-450-TAO metabolite complex, (b) competition between TAO and other substrates for binding to induced cytochrome P-450, or (c) intrinsic hypoactivity of induced species of cytochrome P-450 with substrates other than TAO itself.

Hypoactivity related to the in vivo *formation of an inactive cytochrome P-450-TAO metabolite complex.* In a preceding communication [6], we reported that cytochrome P-450 in microsomes from TAO-treated rats is partly complexed by a TAO metabolite; treatment of the microsomes with 50 μM potassium ferricyanide displaces the metabolite from its complex and regenerates native cytochrome P-450. Whereas addition of 50 μM potassium ferricyanide to the microsomes did not modify monooxygenase activities in control microsomes (Table 2), it increased them in microsomes from rats treated with 4 daily doses of TAO, 1 mmole/kg i.p. daily (Table 2). This observation shows that the *in vivo* formation of an inactive cytochrome P-450-TAO metabolite complex contributes to the hypoactivity of microsomes from TAO-treated rats. However, much evidence suggests that the *in vivo* formation of inactive complexes is not the only mechanism responsible for hypoactivity. (a) Before addition of potassium ferricyanide, the hepatic concentration of uncomplexed cytochrome P-450 in microsomes from TAO-treated rats was similar to that in microsomes from control rats [6]. Despite normal concentrations of uncomplexed cytochrome P-450 and high activity of NADPH–cytochrome *c* reductase [6], monooxygenase activities were dramatically reduced in TAO-treated rats (Table 1). (b) After addition of potassium ferricyanide, cytochrome P-450 in microsomes from rats treated with 4 daily doses of TAO, 1 mmole/kg i.p. daily, was increased by 260 per cent above that in control microsomes and TAO demethylase activity was increased by 670 per cent above that in control microsomes [6]. However, other monooxygenase activities (Table 2), albeit increased from their basal value by the addition of potassium ferricyanide, nevertheless remained similar to, or lower than, those in control microsomes.

In vitro *inhibition unrelated to the formation of complexes. In vitro*, addition of TAO to microsomes not treated with potassium ferricyanide (Table 3) inhibited hexobarbital hydroxylase activity; the per cent inhibition was 10 times higher in microsomes from rats treated with 4 daily doses of TAO than in control microsomes; inhibition occurred without any detectable decrease in the CO-difference spectrum of the dithionite-reduced incubates (Table 3). Uncomplexed cytochrome P-450 of TAO-treated rats is poorly able to demethylate and oxidize TAO into a complex-forming metabolite [6] but gives a 10 times higher type I binding spectrum with TAO than control microsomes [6]. In uncomplexed cytochrome P-450 of TAO-treated rats, TAO may compete with other substrates for the substrate-binding site of cytochrome P-450. According to this view, TAO may inhibit the metabolism of other substrates in at least 2 ways: (a) through competition with other substrates for the substrate-binding site of cytochrome P-450, and (b) through the inactivation of

cytochrome P-450 by the formation of a stable cytochrome P-450-TAO metabolite complex. Such a dual mechanism for inhibition has been already suggested with other compounds transformed into complex-forming metabolites [7]. In TAO-treated rats, significant antibiotic activities were found in the liver at the time of sacrifice, suggesting that competition between the antibiotic remaining in the liver and other substrates may contribute to the *in vivo* decrease in drug metabolism (Table 1, Fig. 1).

Hypoactivity of uncomplexed cytochrome P-450. Uncomplexed cytochrome P-450 of TAO-treated rats resembles cytochrome P-450 of control rats in that it is poorly able to demethylate and oxidize TAO into a complex-forming metabolite [6]. However, uncomplexed cytochrome P-450 of TAO-treated rats differs from cytochrome P-450 of control rats in several other ways: (a) it has a 10 times higher TAO-binding capacity [6]; (b) its inhibition by TAO *in vitro* is 10 times higher (Table 3); (c) it has a markedly reduced activity with substrates (Table 1) other than TAO itself [6]; (d) it has substrate-binding and metabolizing kinetics different from those of cytochrome P-450 from control rats (Fig. 2). These observations suggest that uncomplexed cytochrome P-450 of TAO-treated rats is made up of species of cytochrome P-450 which differ in nature and/or proportion from species present in control rats. These species are characterized by a high overall affinity for TAO but a low overall activity with other substrates. It is not yet known however whether this low activity is an intrinsic property of species of cytochrome P-450 induced by TAO or is an indirect consequence of their high affinity for TAO and their inhibition by TAO which remains in the liver at the time of sacrifice and might stick to the microsomes during their preparation. However, some evidence supports the former mechanism for the decreased hexobarbital hydroxylase activity in microsomes isolated from TAO-treated rats: (a) repeated washings of the microsomes with buffer failed to improve the hexobarbital hydroxylase activity in TAO-treated rats; (b) more convincingly, the reduced hexobarbital hydroxylase activity in TAO-treated rats was mainly due to a decrease in the apparent V_{max} whereas competition with TAO remaining in isolated microsomes would, on the contrary, be expected to produce an increase in the apparent K_m.

It is concluded that the administration of TAO decreases several monoxygenase activities and that hypoactivity is partly related to the *in vivo* formation, in TAO-induced rats, of an inactive cytochrome P-450–TAO metabolite complex. It is suggested that other mechanisms for hypoactivity may include (a) *in vivo* competition between TAO and other substrates for the substrate-binding site of TAO-induced cytochrome P-450, and (b) intrinsic hypoactivity of TAO-induced cytochrome P-450 for some substrates.

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