0022-3565/02/3022-584–593$7.00
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
JPET 302:584–593, 2002

Vol. 302, No. 2
34744/998710
Printed in U.S.A.

Persistent Suppression of Hepatic CYP2A1 Expression and Serum Triiodothyronine Levels by Tamoxifen in Intact Female Rats: Dose-Response Analysis and Comparison with 4-Hydroxytamoxifen, Fulvestrant (ICI 182,780), and 17β-Estradiol-3-benzoate

LUDGER M. ICKENSTEIN[1] and STELVIO M. BANDIERA

Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, British Columbia, Canada

Received February 18, 2002; accepted April 29, 2002

ABSTRACT

Tamoxifen, a nonsteroidal antiestrogen, is used widely in the treatment of breast cancer and is undergoing evaluation as a chemopreventive agent. In this study, we investigated several long-term effects of tamoxifen in intact adult female rats following acute treatment at various dosages. The effects of tamoxifen on somatic growth, growth hormone (GH) levels, thyroid hormone levels, and on hepatic cytochrome P450 (P450) expression were compared with those of fulvestrant (ICI 182,780), 17β-estradiol-3-benzoate, and 4-hydroxytamoxifen under the same experimental conditions. Each compound was injected s.c. for two consecutive days, and rats were killed 37 days after treatment. Tamoxifen decreased body weight and serum triiodothyronine (T3) levels at dosages ranging from 0.5 to 200 mg/kg. Ovary weight, uterus weight, peak plasma GH concentration, and hepatic CYP2A1 content were decreased 37 days after treatment with tamoxifen at a dosage of 20 mg/kg, but expression of other P450 enzymes was not affected. However, tamoxifen and 4-hydroxytamoxifen could not be detected in plasma by high performance liquid chromatography analysis at this time, which suggests that the effects of tamoxifen were mediated indirectly. 4-Hydroxytamoxifen exhibited effects similar to those of tamoxifen, indicating that this metabolite contributes to the in vivo activity of tamoxifen. Estradiol benzoate decreased CYP2A1 and increased CYP3A hepatic levels, but had no effect on serum T3 concentration. In contrast, treatment with ICI 182,780 had little or no effect on the endpoints measured. In summary, 2-day tamoxifen treatment of intact adult female rats resulted in persistent suppression of somatic growth, serum T3 levels, and hepatic CYP2A1 expression.

Tamoxifen is a triphenylethylene derivative that acts as a full estrogen agonist, partial agonist, or antagonist depending on the species and target organ (MacGregor and Jordan, 1998). In human breast tissue, tamoxifen is a competitive antagonist with a binding affinity for the estrogen receptor that is approximately 2% of that of 17β-estradiol (Robertson et al., 1982). Tamoxifen blocks the proliferative stimulation of the mammary gland by estrogen and has become the endocrine therapy of choice for all stages of breast cancer in both premenopausal and postmenopausal women. Recently, tamoxifen has been proposed for prophylactic use in healthy women at high risk for breast cancer (Smith et al., 2000). Like estrogen, tamoxifen helps prevent osteoporosis and spinal fractures in postmenopausal women and lowers serum cholesterol levels. However, tamoxifen treatment slightly increases the risk of thrombosis and pulmonary embolisms (MacGregor and Jordan, 1998; Smith et al., 2000), but the primary concern with long-term tamoxifen use, especially as a chemopreventive agent, is an increased risk of endometrial cancer (Fornander et al., 1989). Novel antiestrogens such as fulvestrant (ICI 182,780) are devoid of estrogen agonist activity and may eventually replace tamoxifen for the treatment of breast cancer. ICI 182,780 is a 7α-alkyl analog of 17β-estradiol that binds the estrogen receptor with approximately the same binding affinity as estradiol and blocks the uterotropic activity of tamoxifen and estradiol in immature female rats (Wakeling et al., 1991).

This work was funded by a grant from the Medical Research Council of Canada (MT-13479).
Results of the study were presented in part at the 38th Annual Meeting of the Society of Toxicology, New Orleans, LA, March 14–18, 1999.
[1] Current address: British Columbia Cancer Agency, 601 West 10th Avenue, Vancouver, British Columbia, Canada V5Z 4E6.
Article, publication date, and citation information can be found at http://jpet.aspetjournals.org.
DOI: 10.1124/jpet.102.034744.

ABBREVIATIONS: P450, cytochrome P450; GH, growth hormone; HPLC, high performance liquid chromatography; PAGE, polyacrylamide gel electrophoresis; T3, triiodothyronine; T4, thyroxine.

In rats, chronic tamoxifen administration leads to a dose-related increase in hepatocellular tumors (Greaves et al., 1993). The mechanism of this hepatocarcinogenic activity by tamoxifen is not fully understood but involves metabolic activation of tamoxifen to a reactive intermediate that binds covalently to proteins and DNA (Dehal and Kupfer, 1999). Hepatic biotransformation of tamoxifen yields tamoxifen *N*-oxide, *N*-desmethyltamoxifen, and 4-hydroxytamoxifen as major metabolites (Mani et al., 1993). Formation of *N*-desmethyltamoxifen and 4-hydroxytamoxifen is catalyzed by the cytochrome P450 (P450) enzymes including CYP2C, CYP2D, and CYP3A (Mani et al., 1993; Dehal and Kupfer, 1999), and experimental evidence suggests that 4-hydroxylation of tamoxifen stabilizes the formation of the reactive intermediate α-hydroxytamoxifen (Potter et al., 1994; Dehal and Kupfer, 1999).

Factors affecting biotransformation pathways of tamoxifen are relevant to both its diverse pharmacological actions and its carcinogenic side effects. In rats, tamoxifen induces CYP2B and CYP3A enzymes, which are involved in its bioactivation and metabolism (White et al., 1993). In addition to the reversible induction effect, tamoxifen produces long-lasting changes in hepatic levels of other P450 enzymes. Studies from our laboratory showed that neonatal tamoxifen treatment resulted in altered hepatic P450 expression in adult rats (Kawai et al., 1999). However, it remains to be determined whether prolonged alteration of P450 expression produced by tamoxifen is restricted to developmental exposure in animals with undeveloped gonads or whether it represents a general effect that also applies to sexually mature animals. Another unresolved question is whether tamoxifen or its active metabolites persist for an extended period after administration and exert effects directly or produce hormonal alterations, which modulate P450 enzyme expression indirectly. Support for a hormonal mechanism stems from reports that tamoxifen suppressed growth hormone (GH) secretion in adult male and female rats for at least 7 weeks after administration (Tannenbaum et al., 1992), interfered with thyroid hormone activity (DiPippo and Powers, 1997; Fitts et al., 1998), and enhanced modulation by exogenous androgen of selected P450 enzymes in adult female rats (Chang et al., 1996). Chronic tamoxifen treatment affects endocrine-mediated responses such as somatic growth, uterine weight, and reproductive success that are dependent on gonadal, pituitary, and thyroid hormones (Bowman et al., 1983; MacGregor and Jordan, 1998). In rats, the sexually dimorphic pattern of GH secretion (Edén, 1979) plays a major regulatory role in the hepatic expression of P450 enzymes such as CYP2A1, CYP2C11, and CYP2C12 (Waxman and Chang, 1995). Therefore, a drug treatment that modifies GH secretion, or interferes with estrogen or thyroid hormones, has the potential to permanently alter pathways of hepatic drug and steroid metabolism.

In the current study, we set out to determine whether acute tamoxifen treatment of intact adult female rats produced prolonged changes in hepatic P450 enzyme expression and whether those changes are accompanied by changes in circulating GH or thyroid hormone levels or are associated with measurable serum concentrations of tamoxifen and 4-hydroxytamoxifen. Additional goals were to directly compare the effects of tamoxifen with those of a pure estrogen antagonist, ICI 182,780, and an estrogen agonist, estradiol benzoate, and to determine whether 4-hydroxytamoxifen contributes to the diverse side effects of the parent compound.

Experimental Procedures

Materials. Tamoxifen (free base), 4-hydroxytamoxifen (70% *Z*-isomer), and 17β-estradiol-3-benzoate were purchased from Sigma-Aldrich (St. Louis, MO). ICI 182,780 was obtained from Tocris Cookson (Ballwin, MO). Other reagents were obtained from sources reported previously (Wong and Bandiera, 1996).

Animals. Adult female Long-Evans rats weighing 175 to 225 g (7–10 weeks of age) were purchased from Charles River Canada (Montreal, QC, Canada). Upon arrival, rats were housed in pairs on corncob bedding in polycarbonate cages with free access to water and Rodent Laboratory Diet 5001 (PMI Feeds Inc., Richmond, IN). Animal quarters were maintained at a temperature of 20–23°C with a 12-h photoperiod. Rats were cared for in accordance with the principles and guidelines of the Canadian Council on Animal Care.

Animal Treatment and Collection of Blood and Tissue Samples. Two acute exposure studies were conducted using a 2-day treatment protocol, which was shown previously to inhibit 7,12-dimethylbenz(a)anthracene-induced tumor growth for more than 4 months after administration (Jordan, 1976). For the dose-response study, rats were divided into seven treatment groups with six animals in each group. After a 3-day acclimation period, rats were injected s.c. with tamoxifen in peanut oil at dosages of 0.5, 5, 20, 100, or 200 mg/kg on two consecutive days. A control group received peanut oil alone at 2 ml/kg. Rats were weighed on each day of treatment and at 11, 21, 31, and 37 days after the start of treatment. Blood samples for the GH assay were collected every 20 min for 8 h (starting at 9:00 AM) on day 34 from three control rats and three rats treated with tamoxifen at dosages of 20 or 200 mg/kg. Blood (250 μl) was drawn from the tip of the tail into heparin-coated glass capillary tubes (Natelson Blood Collecting Tubes; Fisher Scientific, Vancouver, BC, Canada) and placed on ice. Trunk blood was collected after decapitation of rats at 37 days after the start of treatment, placed on ice, and allowed to clot. Serum and plasma were separated by centrifugation at 13,000g for 15 min at 4°C and stored immediately at −75°C until analysis. Rat livers were excised immediately and hepatic microsomes were prepared as described below. In a separate experiment, three intact adult female rats were injected subcutaneously with tamoxifen in peanut oil at 50 mg/kg on two consecutive days, and blood samples for HPLC analysis of tamoxifen and 4-hydroxytamoxifen were collected every 12 days after treatment. Blood (1 ml) was drawn from the tip of the tail into heparin-coated glass capillary tubes, and plasma was separated and stored as described above.

In a second study, rats were divided into six treatment groups with eight animals in each group. After a 3-day acclimation period, rats were injected subcutaneously on two consecutive days with tamoxifen (20 mg/kg), 4-hydroxytamoxifen (20 mg/kg), ICI 182,780 (10 mg/kg), 17β-estradiol-3-benzoate (1 mg/kg), or tamoxifen (20 mg/kg) and 17β-estradiol-3-benzoate (1 mg/kg) given as two separate injections. The dosage of tamoxifen and 4-hydroxytamoxifen selected was based on the results of the dose-response experiment. Smaller dosages were chosen for estradiol benzoate and ICI 182,780 because these compounds have a greater binding affinity for the estrogen receptor (Robertson et al., 1982; Wakeling et al., 1991; MacGregor and Jordan, 1998). Similar dosages were shown previously to maintain (in the case of estradiol benzoate) or alter (in the case of ICI 182,780) uterine weight and other estrogen responses in ovariectomized (ICI 182,780) or intact rats (estradiol benzoate) (Wakeling et al., 1991; Bandiera and Dworschak, 1992). Tamoxifen and estradiol benzoate were dissolved in peanut oil, ICI 182,780 was dissolved in propylene glycol, and 4-hydroxytamoxifen was injected as a suspension in peanut oil. Control rats received peanut oil or propylene

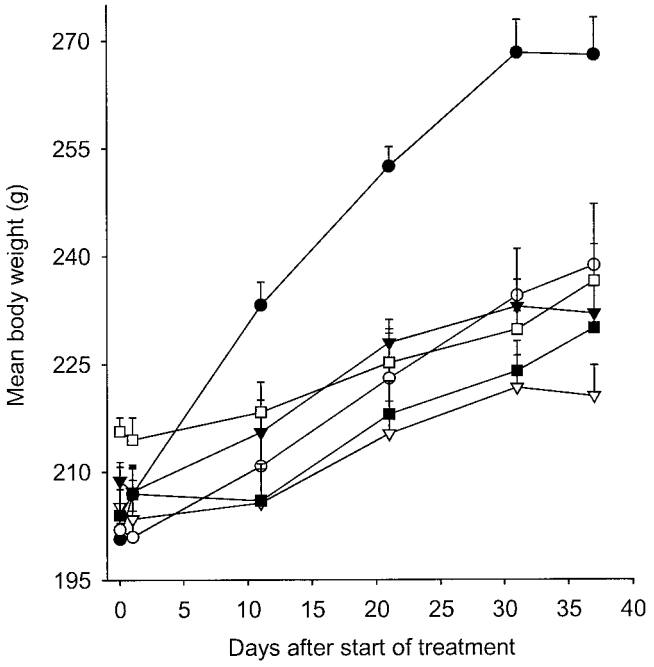

Fig. 1. Body weights of intact adult female rats following treatment with tamoxifen. Rats were injected once daily for two consecutive days with peanut oil (●) or tamoxifen at dosages of 0.5 (○), 5 (▼), 20 (▽), 100 (■), or 200 mg/kg (□). Body weights are presented as the mean ± S.E.M. of six rats per treatment group. Mean body weights of rats treated with tamoxifen at all dosages were significantly different ($p < 0.05$) from those of vehicle-treated rats from 11 to 37 days after the start of treatment.

glycol alone at 2 ml/kg. Food intake was measured every 5 days. Four rats from each treatment group were killed by decapitation at 17 days, and the remaining four animals were killed at 37 days after the start of treatment. Trunk blood was collected, placed on ice, and allowed to clot. Serum was prepared and stored as described above. Liver, uterus, and ovaries were excised after decapitation from each animal, blotted dry, and weighed, and hepatic microsomes were prepared immediately.

Preparation of Hepatic Microsomes and Microsomal Assays. Hepatic microsomes were prepared from individual rats by differential ultracentrifugation. The final microsomal pellet was suspended in 0.25 M sucrose, and aliquots of the suspension were stored at −75°C. Total P450 concentration was determined from the sodium dithionite-reduced carbon monoxide difference spectrum using a molar extinction coefficient of 91 $cm^{-1} \cdot mM^{-1}$, as described previously (Wong and Bandiera, 1996). Microsomal protein concentration and microsomal testosterone hydroxylase activities were determined as reported previously (Anderson et al., 1998).

Preparation of P450 Proteins and Antibodies. Purified rat CYP2A1, CYP2B1, and CYP3A1 were included as calibration standards in the immunoblot assays. Rat CYP2A1 was provided by Dr. A. Parkinson (University of Kansas Medical Center, Kansas City, KS). Rat CYP2B1 and CYP3A1 were purified as reported previously (Wong and Bandiera, 1996; Anderson et al., 1998). Sheep anti-rat CYP2A1 polyclonal IgG was donated by Dr. P. E. Thomas (The State University of New Jersey-Rutgers, Piscataway, NJ). Rabbit anti-rat CYP2B1 polyclonal IgG and rabbit anti-rat CYP3A1 polyclonal IgG were prepared as reported previously (Wong and Bandiera, 1996; Anderson et al., 1998). The antibodies reacted with the following P450 enzymes, but not with other P450 proteins. Sheep anti-CYP2A1 IgG reacted primarily with CYP2A1 but also recognized CYP2A2. Rabbit anti-CYP2B1 IgG reacted equally with rat CYP2B1 and CYP2B2, and recognized a third noninducible member of the CYP2B subfamily. Individual CYP2A and CYP2B proteins can be resolved by SDS-PAGE and were quantified separately. Rabbit anti-CYP3A1 IgG was back-absorbed as reported (Anderson et al., 1998) and reacted predominantly with CYP3A1. It also recognized CYP3A2 and may react with other CYP3A enzymes that cannot be resolved from CYP3A1 by SDS-PAGE.

SDS-PAGE and Immunoblot Assay. SDS-PAGE was performed as reported previously (Wong and Bandiera, 1996). Proteins resolved by SDS-PAGE were transferred electrophoretically onto nitrocellulose membranes. Membranes were incubated for 2 h at 37°C with sheep anti-CYP2A1 IgG at 10 μg of IgG/ml, rabbit anti-CYP2B1 IgG at 2 μg of IgG/ml, or rabbit anti-CYP3A1 IgG at 50 μg of IgG/ml. Membranes were then washed and incubated for 2 h at 37°C with alkaline phosphatase-linked rabbit $F(ab')_2$ anti-sheep IgG (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, MD) at a dilution of 1:1000, or with alkaline phosphatase-linked goat $F(ab')_2$ anti-rabbit IgG (Tago BioSource International, Camarillo, CA) at a dilution of 1:3000. Assay conditions were optimized to ensure that color development did not proceed beyond the linear response range of the phosphatase reaction. Staining intensities of protein bands were determined using a pdi 420oe densitometer equipped with an AGFA Arcus II scanner and the pdi Quantity One 3.0 software (pdi Inc., Huntington Station, NY). The amount of immunoreactive protein was determined from the ratio of the integrated intensity of the stained band to that of the internal standard. Values of integrated intensity were converted into picomoles by means of calibration curves prepared with purified rat P450 standards. The CYP3A protein level is expressed as the optical density (OD × mm^2) of the stained band relative to the optical density of an internal standard because CYP3A1 was the only purified CYP3A enzyme available for use as a calibration standard, and anti-CYP3A1 IgG may react with other CYP3A.

Growth Hormone and Thyroid Hormone Assays. Plasma GH concentration was measured with an enzyme immunoassay kit containing rat GH-biotin conjugate, goat anti-rat GH serum, and donkey anti-goat IgG-coated microtiter plates (Amersham Biosciences, Baie d'Urfé, QC, Canada). Assays were performed as described by the manufacturer. Absorbance was measured at 450 nm with a spectrophotometric microplate reader (model EL 309; Bio-Tek Instruments, Winooski, VT). GH plasma concentrations were derived from GH standards included on each plate. The limit of detection of the assay was reported by the manufacturer to be approximately 2 ng/ml and the cross-reactivity of the goat anti-GH IgG to be less than 0.3% for rat thyroid-stimulating hormone, luteinizing hormone, prolactin, follicle-stimulating hormone, and adrenocorticotropic hormone.

Total serum 3,3′,5-triiodothyronine (T3) concentrations were measured with a solid phase ^{125}I radioimmunoassay kit containing rabbit anti-T3 polyclonal IgG-coated test tubes and a radioactive T3 tracer solution (ICN Biomedicals, Costa Mesa, CA). The limit of detection of the assay was reported by the manufacturer to be approximately 0.7 ng/ml (1 pmol/ml). The cross-reactivity of the rabbit anti-T3 IgG was reported to be less than 0.18% for L-thyroxine, 0.44% for 3,5-diiodothyronine, and 0.01% for 3,5-diiodothyronine and 3,3′,5′-triiodothyronine. Total serum L-thyroxine (T4) concentrations were measured with a solid phase ^{125}I radioimmunoassay kit containing mouse anti-T4 monoclonal IgG-coated test tubes and a radioactive T4 tracer solution (ICN Biomedicals). The limit of detection of the assay was reported by the manufacturer to be approximately 8 ng/ml (10 pmol/ml). The cross-reactivity of the mouse anti-T4 monoclonal IgG was reported to be less than 31% for D-thyroxine, 1% for T3, 0.002% for 3,5-diiodo-L-thyronine, 3,5-diiodo-L-thyrosine, and 3-iodo-L-thyrosine.

HPLC Analysis of Tamoxifen and 4-Hydroxytamoxifen. The HPLC assay for tamoxifen and 4-hydroxytamoxifen was derived from published methods (MacCallum et al., 1996) with clomifene as the internal standard. Tamoxifen and 4-hydroxytamoxifen were extracted twice from 250 μl of plasma with 3 ml of *n*-butanol/hexane (2:98, v/v). The solvent was evaporated and the residue was reconstituted in 200 μl of methanol and filtered into autosampler vials. Tamoxifen, 4-hydroxytamoxifen, and clomifene were separated at

40°C on a Supelco LC-18 (3 μm, 15 cm × 4.6 mm i.d.) reverse phase column (Supelco, Bellefonte, PA). The column was eluted at flow rate of 0.75 ml/min with an isocratic mobile phase consisting of methanol/water/triethylamine (80:19.9:0.1, v/v) and monitored at 243 nm. Tamoxifen and 4-hydroxytamoxifen peaks were identified by comparison of their retention times with those of authentic standards. Tamoxifen and 4-hydroxytamoxifen were quantified from calibration curves of the ratio of peak area of the authentic standard to that of the internal standard plotted against the concentration of the authentic standard. The limit of detection of the assay was approximately 0.2 nmol/ml for tamoxifen and 0.02 nmol/ml for 4-hydroxytamoxifen.

Statistical Analysis. Differences between the mean value of the peanut oil control group and multiple treatment groups were tested by one-way analysis of variance, followed by the Student-Newman-Keuls test. Differences between the mean value of the propylene glycol control group and the ICI 182,780 treatment group were analyzed by Student's t test. Mean differences with $p < 0.05$ were considered to be statistically significant.

Results

Dose-Response Study. To evaluate the long-term effects of tamoxifen in intact adult rats, we initially conducted a dose-response study using a treatment regimen that was associated with prolonged antineoplastic activity (Jordan, 1976) and altered GH secretion (Tannenbaum et al., 1992).

Body Weights. Tamoxifen administered once daily for two consecutive days at dosages ranging from 0.5 to 200 mg/kg per day had a pronounced and long-lasting effect on body weight in intact adult female rats. Mean body weights of tamoxifen-treated rats were consistently less than those of vehicle-treated rats from 11 to 37 days after the start of treatment, and final body weights of tamoxifen-treated rats were 12 to 22% less than those of vehicle-treated rats (Fig. 1). The effect was not dosage-dependent within the investigated range and corresponded to an initial loss in body weight during the treatment period followed by attenuated weight gain, which persisted for more than 4 weeks after the start of treatment. Liver weight was not affected following treatment with tamoxifen (data not shown).

Hepatic Microsomal P450 Activities and Content. Exposure to tamoxifen at critical early stages of development was shown previously to produce altered hepatic P450 enzyme expression in adult rats (Chang et al., 1996; Kawai et al., 1999). In the present study, we measured the effect of several dosages of tamoxifen on individual hepatic microsomal P450 enzyme levels and several P450-mediated testosterone hydroxylase activities, which serve as catalytic markers of individual P450 enzymes. Testosterone 7α-hydroxylase activity is an enzyme-selective marker for CYP2A1, testosterone 2β- and 6β-hydroxylase activities serve as catalytic indicators of CYP3A, and testosterone 16β-hydroxylase activity is a marker for CYP2B enzymes (Waxman and Chang, 1995). The total P450 content was not significantly different between dosage groups. Following tamoxifen treatment at dosages of 20 mg/kg or greater, testosterone 7α-hydroxylase activity, the major testosterone-metabolizing activity in female rat liver, was decreased by approximately 40% relative to the control group, indicating reduced hepatic CYP2A1 expression (Table 1). Testosterone 2β-, 6β-, 16α-, and 16β-hydroxylase activities, and the rate of formation of androstenedione were unaffected by tamoxifen at all dosages investigated, suggesting that tamoxifen had no effect on hepatic expression of CYP2B, CYP2C, and CYP3A enzymes.

Determination of hepatic CYP2A1, CYP2B, and CYP3A protein levels by immunoblot analysis with specific antibodies and densitometric quantification confirmed that CYP2A1 levels were approximately 35% lower in rats treated with tamoxifen at dosages of 20 mg/kg or greater, relative to control values (Fig. 2). In contrast, administration of tamoxifen had no effect on CYP3A (Fig. 2) or CYP2B levels (data not shown).

GH and Thyroid Hormone Levels. Tannenbaum et al. (1992) had reported that GH secretion was suppressed to baseline levels in adult female rats for up to 7 weeks after administration of tamoxifen for 2 days at a dosage of 5 mg/rat. To determine whether a similar effect of tamoxifen might explain the attenuation in weight gain and suppression of hepatic CYP2A1 expression observed in the present study, GH concentrations were determined in three vehicle-treated rats and three rats treated with tamoxifen at dosages of 20 or 200 mg/kg. Blood samples were collected every 20 min over an 8-h period, 3 days before rats were killed. Individual representative 8-h plasma GH profiles of a control and a tamoxifen-treated rat (20 mg/kg) are presented in Fig. 3. The plasma GH profiles of rats treated with vehicle or tamoxifen at dosages of 20 or 200 mg/kg were similar and typical of adult female rats (Edén, 1979). The plasma profile was characterized by frequent low amplitude pulses separated by trough levels of not less than 20 ng/ml. Average and nadir GH levels were not affected by administration of tamoxifen at dosages of 20 or 200 mg/kg, but the peak GH level of the 20 mg/kg treatment group was lower by approximately 40% relative to the control group. However, the peak GH level was not changed significantly in rats treated at a dosage of 200 mg/kg. There was no obvious correlation between

TABLE 1

Effect of tamoxifen on hepatic microsomal testosterone hydroxylase activities

Rats were injected once daily for two consecutive days with peanut oil or tamoxifen at the dosages indicated. Rats were killed and hepatic microsomes were prepared 37 days after the start of treatment. Enzyme activities are expressed as mean ± S.E.M. of six rats per treatment group, except for the 100 mg/kg dosage group where $n = 5$.

Dosage	Total P450 Content	Testosterone Metabolite					
		2β	6β	7α	16α	16β	Androstenedione
mg/kg	*nmol/mg*	*pmol formed/min/nmol total P450*					
0	0.91 ± 0.03	51 ± 13	294 ± 50	1570 ± 175	121 ± 16	65 ± 7	389 ± 56
0.5	0.95 ± 0.05	62 ± 15	316 ± 37	1360 ± 44	124 ± 7	67 ± 6	372 ± 45
5	1.02 ± 0.02	54 ± 11	331 ± 59	1270 ± 189	129 ± 25	65 ± 11	359 ± 68
20	0.94 ± 0.02	42 ± 5	356 ± 37	934 ± 116*	140 ± 12	80 ± 8	479 ± 57
100	0.82 ± 0.03	43 ± 4	407 ± 27	938 ± 75*	145 ± 15	98 ± 15	515 ± 91
200	0.81 ± 0.03	39 ± 6	350 ± 29	907 ± 88*	112 ± 11	73 ± 9	429 ± 71

* Significantly different from the vehicle-treated group ($p < 0.05$).

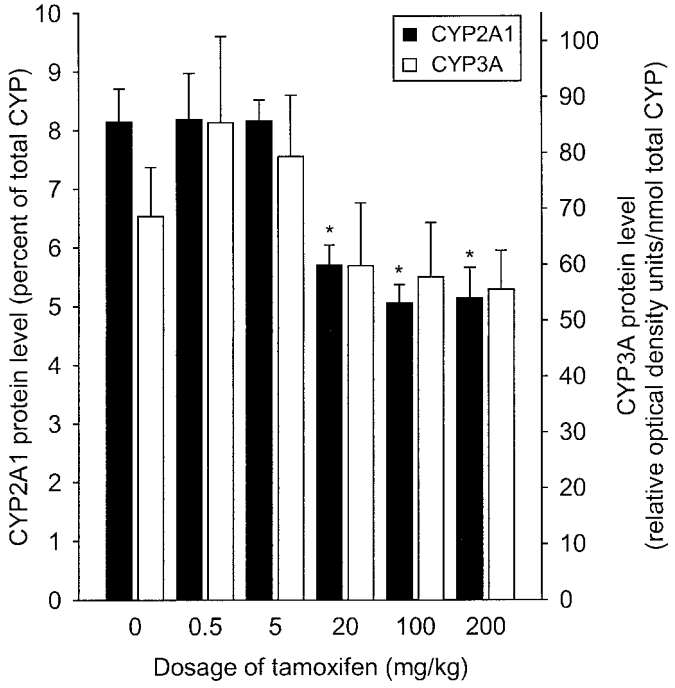

Fig. 2. Hepatic microsomal levels of CYP2A1 and CYP3A in intact adult female rats following treatment with tamoxifen. Hepatic microsomes were prepared 37 days after the start of treatment, and CYP2A1 (left *y*-axis and solid bars) and CYP3A (right *y*-axis and open bars) protein levels were measured by densitometric analysis of immunoblots probed with polyclonal antibodies against CYP2A1 or CYP3A as described under *Experimental Procedures*. Results are presented as the mean ± S.E.M. of six rats per treatment group, except for the 100 mg/kg dosage group where $n = 5$. ⋆, significantly different from the vehicle-treated group ($p < 0.05$).

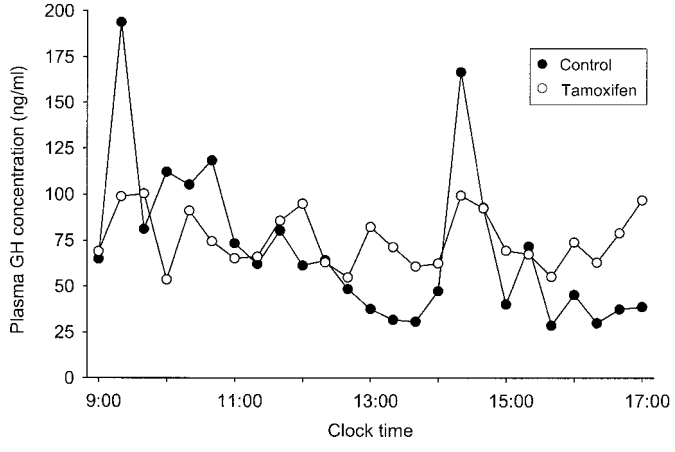

Fig. 3. Representative 8-h plasma GH concentrations following treatment with peanut oil (●) or tamoxifen at a dosage of 20 mg/kg (○). Intact adult female rats were treated once daily for two consecutive days and blood samples (250 μl) were taken every 20 min for 8 h at 34 days after the start of treatment from three rats per treatment group. Data from one rat in each treatment group are shown in the figure.

changes in weight gain or hepatic CYP2A1 levels and diminution of GH secretion by tamoxifen.

To determine whether the attenuated weight gain of tamoxifen-treated rats could be attributed to persistent changes in serum levels of thyroid hormones, T3 and T4 serum levels were measured 37 days after the start of treatment. Mean serum T3 concentrations of rats treated with tamoxifen at all dosages, except 20 mg/kg, were decreased by 28 to 46% relative to control values (Fig. 4A). In contrast, serum T4 concentrations did not change significantly following administration of tamoxifen, except for rats treated at a dosage of 20 mg/kg (Fig. 4B). The discordant results obtained for the 20 mg/kg treatment group may be unreliable because the T3 level was decreased significantly and the T4 level was not increased significantly in rats treated with tamoxifen at this dosage in the second phase of the study (Fig. 7).

Plasma Levels of Tamoxifen and 4-Hydroxytamoxifen. Tamoxifen and its active metabolite 4-hydroxytamoxifen have relatively long biological half-lives in rats of approximately 10 h and 17 h, respectively (Robinson et al., 1991). To investigate the possibility that the long-lasting physiological effects observed after tamoxifen treatment were caused by tamoxifen or 4-hydroxytamoxifen persisting in treated animals, plasma from three of the six control rats and three rats treated with tamoxifen at a dosage of 50 mg/kg was collected at four time points and analyzed by HPLC. Tamoxifen was present in rat plasma at a concentration of 0.75 ± 0.07 μM at 2 days after the start of treatment, de-

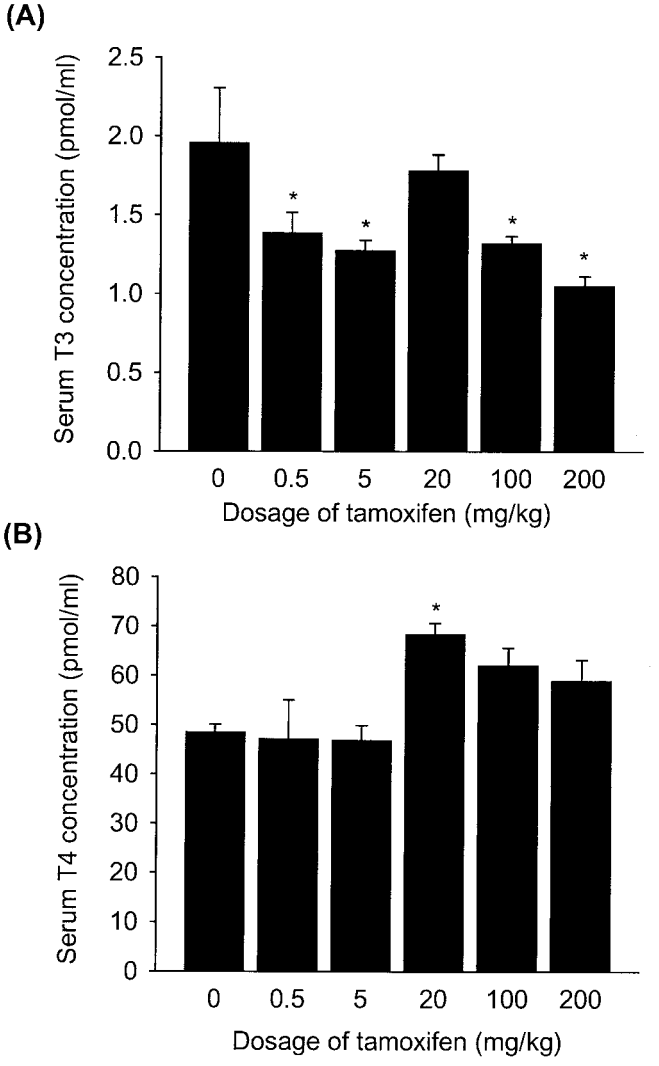

Fig. 4. Serum T3 (A) and T4 (B) levels of intact adult female rats following treatment with tamoxifen. Serum for T3 and T4 determination was collected 37 days after the start of treatment. Results are presented as the mean ± S.E.M. of six rats per treatment group. ⋆, significantly different from the vehicle-treated group ($p < 0.05$).

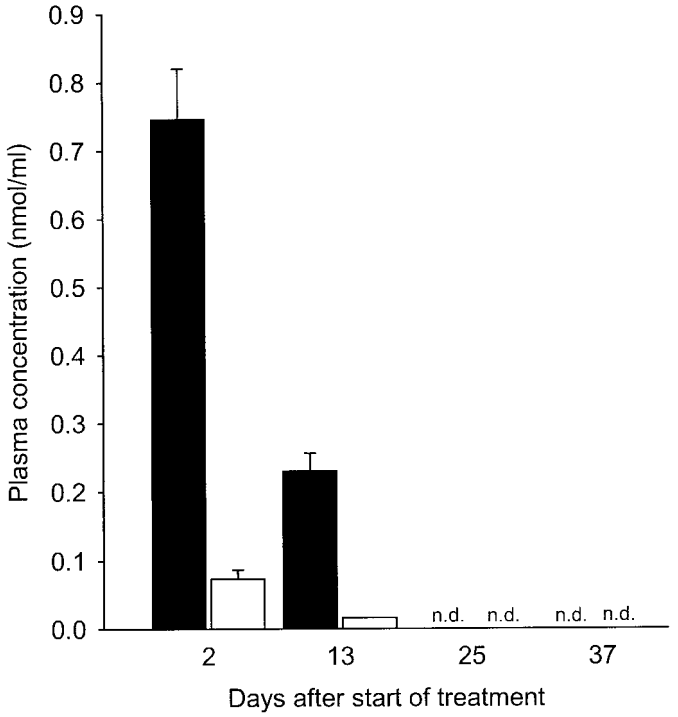

Fig. 5. Plasma concentrations of tamoxifen (solid bars) and 4-hydroxytamoxifen (open bars) at various times after tamoxifen treatment. Intact adult female rats were injected once daily for two consecutive days with tamoxifen at a dosage of 50 mg/kg. Plasma concentrations were determined by HPLC analysis and are presented as the mean ± S.E.M. of three rats per treatment group. At day 12, 4-hydroxytamoxifen was detected in plasma from one rat only. n.d., peaks corresponding to tamoxifen or 4-hydroxytamoxifen could not be detected.

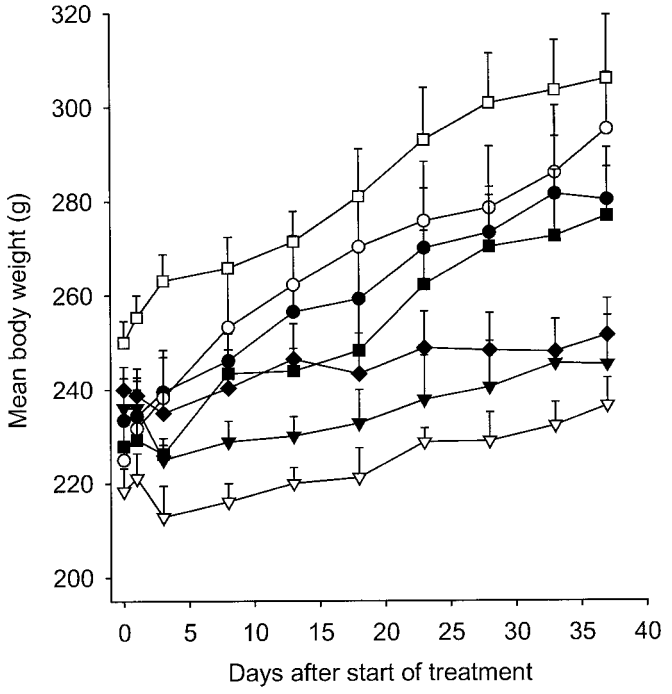

Fig. 6. Body weights of intact adult female rats following treatment with propylene glycol at 2 ml/kg (○), ICI 182,780 at 10 mg/kg (□), peanut oil at 2 ml/kg (●), estradiol benzoate at 1 mg/kg (■), tamoxifen at 20 mg/kg (▽), tamoxifen at 20 mg/kg plus estradiol benzoate at 1 mg/kg (◆), or 4-hydroxytamoxifen at 20 mg/kg (▼). Body weights are presented as the mean ± S.E.M. of eight rats per treatment group for days 0, 1, 3, 8, and 13 and four rats per treatment group for days 18, 23, 28, 33, and 36, except for the tamoxifen plus estradiol benzoate treatment group where $n = 9$ and $n = 5$ rats, respectively.

clined to 0.23 ± 0.03 μM after 13 days, and was no longer detectable at 25 or 37 days after the start of treatment (Fig. 5). 4-Hydroxytamoxifen was present in plasma at approximately 10% of the level of tamoxifen at two days after the start of treatment. At 13 days after the start of treatment, 4-hydroxytamoxifen was detectable at a concentration of 0.02 μM in plasma of one rat only and was no longer detected in any of the rats at 25 or 37 days after the start of treatment (Fig. 5). The results indicate that the effects of tamoxifen on weight gain, serum T3 concentrations, and hepatic CYP2A1 levels were independent of measurable tamoxifen or 4-hydroxytamoxifen plasma concentrations.

Comparison with Estradiol Benzoate, ICI 182,780, and 4-Hydroxytamoxifen. In the second phase of the study, the effects of tamoxifen on growth, thyroid hormone levels, and hepatic P450 expression were compared with those of estradiol benzoate, an estrogen agonist, and the pure steroidal antiestrogen, ICI 182,780, to determine whether the responses were unique to tamoxifen or shared by pure estrogens or antiestrogens. Coadministration of estradiol benzoate with tamoxifen was included to determine whether the effects of tamoxifen could be reversed or enhanced by estradiol benzoate.

Body Weight and Food Intake. Mean body weights of rats treated with estradiol benzoate or ICI 182,780 were essentially the same as those of the respective control groups for up to 37 days after treatment (Fig. 6). The body weight profile of rats treated with ICI 182,780 was elevated in comparison with the propylene glycol group because the initial body weight of the ICI 182,780-treated group was greater, but the overall weight gain of ICI 182,780-treated rats was the same as that of vehicle-treated rats. Administration of tamoxifen and 4-hydroxytamoxifen elicited similar effects and caused an initial loss in body weight followed by reduced weight gain compared with the control group for 37 days after the start of treatment. Coadministration of estradiol benzoate with tamoxifen had a mixed effect. The mean body weight of rats in this treatment group increased to the same extent as that of control rats during the initial 2 weeks after treatment but did not increase thereafter (Fig. 6).

The decreased weight gain of rats treated with tamoxifen, 4-hydroxytamoxifen, or tamoxifen plus estradiol benzoate was associated with decreased food intake (Table 2). The mean relative daily food intake of rats treated with tamoxifen or 4-hydroxytamoxifen was significantly less than that of the control group for the first 13 days after the start of treatment. A similar effect was observed for the first 8 days after the start of treatment for rats treated with tamoxifen plus estradiol benzoate and for the first 3 days after the start of treatment for estradiol benzoate-treated rats. In contrast, administration of ICI 182,780 had no effect on daily food intake. There was no apparent difference in food efficiency (i.e., the ability to convert food consumed into body weight) between treatment groups. Thus, the effects of tamoxifen, 4-hydroxytamoxifen, or tamoxifen plus estradiol benzoate on body weight were attributed to the suppression of food intake.

Hepatic Microsomal P450 Content. Total P450 content of hepatic microsomes prepared 37 days after the start of

TABLE 2

Effect of ICI 182,780, estradiol benzoate, tamoxifen, and 4-hydroxytamoxifen on daily food intake and food efficiency

Rats were injected once daily for two consecutive days with propylene glycol (2 ml/kg), ICI 182,780 (10 mg/kg), peanut oil (2 ml/kg), estradiol benzoate (1 mg/kg), tamoxifen (20 mg/kg), tamoxifen (20 mg/kg) plus estradiol benzoate (1 mg/kg), or 4-hydroxytamoxifen (20 mg/kg), and killed 37 days after the first injection. Food intake was determined by measuring the amount of food provided to each cage (two rats per cage) and the amount remaining at the next time point. Daily food intake is presented as the mean ± S.E.M. of eight rats per treatment group for days 1, 3, 8, and 13 after the first injection, and four rats per treatment group for days 17 and 37, except for the tamoxifen plus estradiol benzoate treatment group where $n = 9$ and $n = 5$, respectively. Mean food efficiency ± S.E.M., calculated as daily weight gain (grams) divided by daily food intake (grams), is shown in parentheses. Food intake and food efficiency were also determined for each treatment group on days 23, 28, and 33, but values were not different from those listed for days 18 and 37 and thus are not included in the table.

Treatment	Daily Food Intake (mg/g body wt) and (Food Efficiency) (g wt gain/g food intake)					
	Day 1	Day 3	Day 8	Day 13	Day 18	Day 37
Propylene glycol	74.6 ± 3.6 (0.39 ± 0.06)	83.4 ± 0.3 (0.13 ± 0.07)	79.3 ± 5.8 (0.09 ± 0.05)	67.6 ± 3.6 (0.09 ± 0.08)	67.3 ± 0.3 (0.06 ± 0.04)	57.2 ± 1.3 (0.11 ± 0.09)
ICI 182,780	75.0 ± 3.6 (0.3 ± 0.07)	81.4 ± 3.4 (0.2 ± 0.01)	72.1 ± 1.7 (0.03 ± 0.03)	71.2 ± 3.0 (0.06 ± 0.03)	73.1 ± 10.0 (0.05 ± 0.02)	63.5 ± 4.2 (0.04 ± 0.01)
Peanut oil	71.8 ± 2.5 (0.06 ± 0.15)	68.9 ± 3.2 (0.16 ± 0.02)	71.9 ± 2.9 (0.07 ± 0.04)	69.6 ± 0.7 (0.12 ± 0.01)	64.7 ± 8.4 (−0.03 ± 0.06)	58.8 ± 4.2 (−0.02 ± 0.04)
Estradiol benzoate	59.4 ± 5.9 (0.08 ± 0.13)	53.9 ± 1.8* (−0.13 ± 0.07)	72.3 ± 3.7 (0.14 ± 0.03)	72.3 ± 1.6 (0.05 ± 0.04)	75.4 ± 1.7 (0.08 ± 0.01)	66.6 ± 3.9 (0.06 ± 0.05)
Tamoxifen	55.3 ± 2.2* (0.23 ± 0.1)	40.0 ± 6.6* (−0.72 ± 0.46)	57.5 ± 3.4* (0.04 ± 0.06)	61.1 ± 2.8* (0.05 ± 0.06)	62.7 ± 3.4 (0.01 ± 0.01)	63.4 ± 0.5 (0.07 ± 0.03)
Tamoxifen + Estradiol benzoate	45.0 ± 5.4* (−0.17 ± 0.15)	47.5 ± 7.0* (−0.18 ± 0.03)	53.5 ± 2.3* (0.08 ± 0.03)	64.1 ± 1.6 (0.08 ± 0.01)	63.3 ± 0.8 (0.02 ± 0.04)	61.4 ± 8.8 (0.08 ± 0.21)
4-Hydroxytamoxifen	54.2 ± 3.4* (−0.01 ± 0.09)	37.0 ± 2.6* (−0.69 ± 0.13)	50.4 ± 2.4* (0.06 ± 0.02)	61.3 ± 2.0* (0.02 ± 0.02)	63.6 ± 0.6 (0.06 ± 0.01)	56.3 ± 0.5 (0.00 ± 0.08)

* Significantly different from the vehicle-treated group ($p < 0.05$).

treatment was not significantly different among treatment groups. The hepatic CYP2A1 protein level was decreased by approximately 25% for rats treated with estradiol benzoate or tamoxifen (Fig. 7). CYP2A1 protein levels were also decreased following administration of 4-hydroxytamoxifen, but the change was not significant. Administration of ICI 182,780 or the combined treatment with tamoxifen and estradiol benzoate had no effect on hepatic CYP2A1 protein levels. Hepatic CYP3A levels were elevated significantly after administration of estradiol benzoate or coadministration of tamoxifen with estradiol benzoate (Fig. 7). Administration of tamoxifen, 4-hydroxytamoxifen, or ICI 182,780 had no effect on hepatic CYP3A protein levels. Expression of CYP2B enzymes was not affected by any of the treatments (data not shown).

Uterus and Ovary Weights. Uterus and ovary weights were measured at 17 and 37 days after the start of treatment. Because changes in body weight can potentially complicate interpretation of organ weight findings, absolute and relative organ weights are presented in Table 3. Absolute and relative uterus weights were decreased by more than 50% at 37 days after the start of treatment with tamoxifen, 4-hydroxytamoxifen, or tamoxifen plus estradiol benzoate, whereas administration of estradiol benzoate or ICI 182,780 had no effect. Absolute ovary weight was decreased by 20 to 40% following treatment with tamoxifen, 4-hydroxytamoxifen, and tamoxifen plus estradiol benzoate. A small decrease in relative ovary weight was observed following treatment with ICI 182,780. Similar but smaller changes in uterus weight were noted at 17 days after the start of treatment with tamoxifen or 4-hydroxytamoxifen. The results demonstrate that the effect of tamoxifen on uterus and ovary weights is more severe at the later time point, distinct from that of estradiol benzoate and ICI 182,780, and could not be prevented or reversed by coadministration of estradiol benzoate.

Serum T3 and T4 Levels. Serum T3 levels were decreased by approximately 25% at 37 days after administration of tamoxifen at a dosage of 20 mg/kg or coadministration of tamoxifen plus estradiol benzoate (Fig. 8A). Following treatment with ICI 182,780, mean serum T3 levels were increased slightly but significantly relative to the vehicle-treated group. None of the treatments affected serum T4 levels (Fig. 8B).

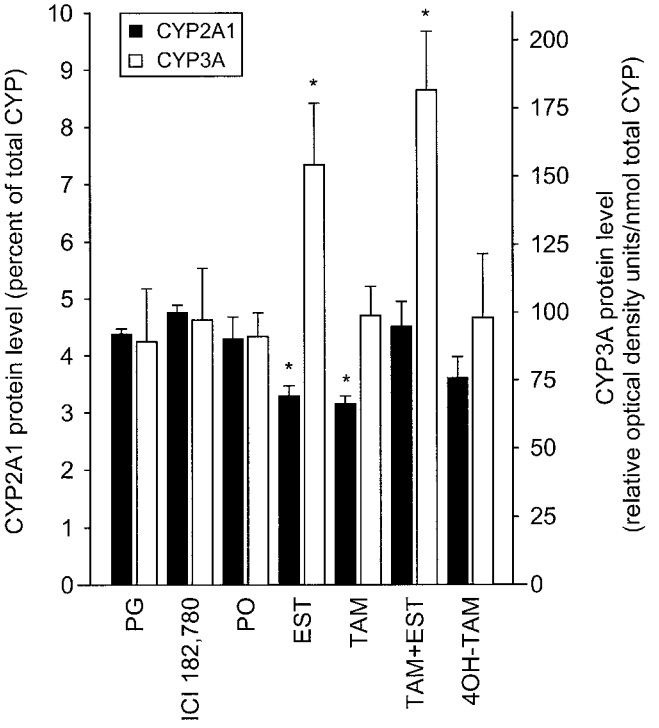

Fig. 7. Hepatic microsomal levels of CYP2A1 and CYP3A in intact adult female rats following treatment with propylene glycol (PG), ICI 182,780, peanut oil (PO), estradiol benzoate (EST), tamoxifen (TAM), tamoxifen and estradiol benzoate together (TAM+EST), or 4-hydroxytamoxifen (4OH-TAM). Hepatic microsomes were prepared 37 days after the start of treatment, and CYP2A1 (left *y*-axis and solid bars) and CYP3A (right *y*-axis and open bars) protein levels were measured by densitometric analysis of immunoblots probed with polyclonal antibodies against CYP2A1 or CYP3A as described under *Experimental Procedures*. Results are presented as the mean ± S.E.M. of four rats per treatment group, except for the tamoxifen plus estradiol benzoate treatment group where $n = 5$. ⋆, significantly different from the vehicle-treated group ($p < 0.05$).

TABLE 3

Effect of ICI 182,780, estradiol benzoate, tamoxifen, and 4-hydroxytamoxifen on uterus and ovary weights

Rats were injected once daily for two consecutive days with vehicle or drug, and organ wet weights were measured 17 and 37 days after the first injection. For dosages, see Table 2. Results are presented as mean ± S.E.M. of four rats per treatment group, except for the tamoxifen plus estradiol benzoate treatment group where $n = 5$ at day 37. Organ weights expressed as a percentage of body weight are shown in parentheses.

Treatment	Day 17		Day 37	
	Uterus Weight	Ovary Weight	Uterus Weight	Ovary Weight
	g		*g*	
Propylene glycol	N.D.	N.D.	0.43 ± 0.01 (0.15 ± 0.006)	0.11 ± 0.01 (0.037 ± 0.002)
ICI 182,780	0.28 ± 0.04 (0.10 ± 0.013)	0.10 ± 0.001 (0.038 ± 0.003)	0.39 ± 0.02 (0.13 ± 0.005)	0.09 ± 0.01 (0.030 ± 0.001)*
Peanut oil	0.36 ± 0.05 (0.14 ± 0.019)	0.14 ± 0.01 (0.053 ± 0.005)	0.41 ± 0.02 (0.15 ± 0.009)	0.10 ± 0.01 (0.037 ± 0.002)
Estradiol benzoate	0.28 ± 0.04 (0.11 ± 0.015)	0.12 ± 0.01 (0.048 ± 0.003)	0.40 ± 0.02 (0.15 ± 0.009)	0.09 ± 0.00 (0.032 ± 0.001)
Tamoxifen	0.16 ± 0.01* (0.072 ± 0.003)*	0.11 ± 0.01 (0.048 ± 0.003)	0.16 ± 0.01* (0.070 ± 0.003)*	0.07 ± 0.01* (0.030 ± 0.002)*
Tamoxifen + Estradiol benzoate	0.25 ± 0.04 (0.096 ± 0.011)	0.09 ± 0.01* (0.035 ± 0.002)*	0.17 ± 0.01* (0.067 ± 0.004)*	0.08 ± 0.00* (0.030 ± 0.001)*
4-Hydroxytamoxifen	0.16 ± 0.01* (0.069 ± 0.004)*	0.11 ± 0.00 (0.046 ± 0.002)	0.16 ± 0.01* (0.066 ± 0.003)*	0.06 ± 0.00* (0.026 ± 0.001)*

N.D., not determined.

* Significantly different from the vehicle-treated group ($p < 0.05$).

Discussion

Previous studies have shown that tamoxifen treatment during the neonatal period alters adult expression of hepatic CYP2A1, CYP2C11, and CYP3A9 enzymes in rats (Kawai et al., 1999), whereas pubertal exposure to tamoxifen enhances the masculinizing effect of exogenous androgen on CYP2C11 and CYP3A2 in adult female rats (Chang et al., 1996). The present study establishes that long-lasting selective alteration of hepatic P450 expression is not restricted to developmental exposure but occurs with tamoxifen treatment of sexually mature animals.

In general, the effects of adult tamoxifen treatment on P450 enzyme expression were similar to those of neonatal tamoxifen treatment, but some differences were apparent. Neonatal administration of tamoxifen decreased CYP2A1-mediated testosterone 7α-hydroxylase activity, CYP3A-mediated testosterone 6β-hydroxylase activity, and CYP3A9 mRNA expression in adult female rats (Kawai et al., 1999). In the present study, acute treatment of intact adult female rats with tamoxifen decreased testosterone 7α-hydroxylase activity and hepatic CYP2A1 content but had no effect on testosterone 6β-hydroxylase activity or CYP3A protein levels. As in the earlier study, tamoxifen had no effect on CYP2B1 or CYP2B2 enzyme expression, indicating that the direct inductive effect of tamoxifen (White et al., 1993) does not persist for 5 weeks after treatment, possibly because circulating and hepatic levels of tamoxifen do not remain sufficiently high. In fact, the observation that neither tamoxifen nor 4-hydroxytamoxifen was detected in rat plasma at 25 and 37 days after the start of treatment implies that the decrease in CYP2A1 content was mediated indirectly. CYP2A1 is sexually and developmentally regulated in rats. The precise hormonal mechanism for the female-predominant expression of this enzyme is not understood completely, but the sexually dimorphic pattern of GH secretion appears to play a major role (Waxman et al., 1989), although estrogen (Waxman et al., 1989) and thyroid hormones (Yamazoe et al., 1990; Waxman and Chang, 1995) also influence CYP2A1 regulation. Relatively little is known about the hormonal regulation of CYP3A9, which is the only rat CYP3A enzyme identified to date that exhibits female-predominant expression (Kawai et al., 2000). To test the possibility that a mechanism related to the estrogen agonist or antagonist of tamoxifen was involved, the effects of tamoxifen were compared with those of estradiol benzoate and ICI 182,780. Treatment with estradiol benzoate decreased hepatic CYP2A1 and increased CYP3A expression, whereas ICI 182,780 had no effect on CYP2A1 or CYP3A protein levels. Moreover, the effects of tamoxifen were not enhanced or reversed by coadministration of tamoxifen with estradiol benzoate. Thus, the results do not support the hypothesis that the suppressive effect of tamoxifen on CYP2A1 expression was estrogen-mediated.

The sustained reduction in weight gain and food intake observed in the present study after acute treatment with tamoxifen or 4-hydroxytamoxifen has not been reported previously for intact rats. However, the results are consistent with previous studies in which weight gain and food intake were suppressed in ovariectomized rats treated with tamoxifen by daily injections (Wade and Heller, 1993) or following a single administration (Bowman et al., 1983). In this respect, tamoxifen would appear to mimic estrogen. The effects of estrogens on food intake, exercise, and somatic growth of rats are well known and are mediated by a central action on the hypothalamus (Tartellin and Gorski, 1971; Wade and Gray, 1979; Hart, 1990). However, tamoxifen is weakly estrogenic, and neither tamoxifen nor 4-hydroxytamoxifen were detectable in plasma by 25 days after treatment, which suggests that growth suppression is unlikely to be the result of estrogenic activity. In the present study, administration of estradiol benzoate did not alter body weight, although food intake was temporarily reduced. Similar results have been reported for estrogen preparations in gonadally intact female rats (Valette et al., 1980: Bandiera and Dworschak, 1992), signifying that exogenous estrogens are relatively ineffective in the presence of endogenous estrogen secretion. Nevertheless, suppression of weight gain can occur with prolonged treatment with exogenous estrogens (Biegel et al., 1998).

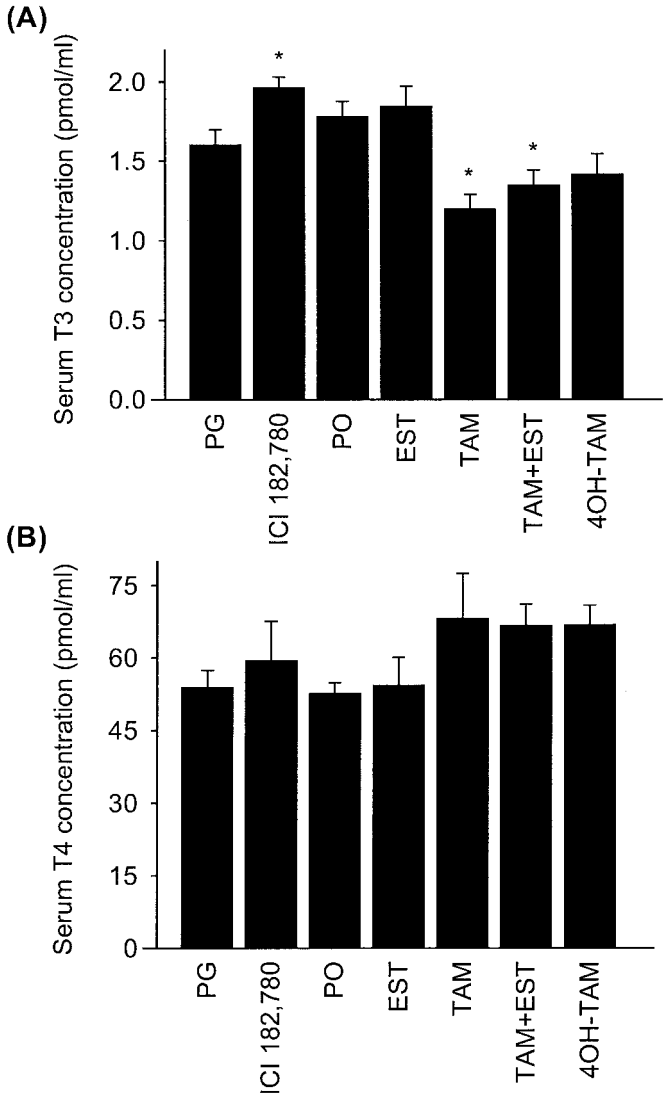

Fig. 8. Serum T3 (A) and T4 (B) levels in intact adult female rats following treatment with propylene glycol (PG), ICI 182,780, peanut oil (PO), estradiol benzoate (EST), tamoxifen (TAM), tamoxifen plus estradiol benzoate (TAM+EST), or 4-hydroxytamoxifen (4OH-TAM). Serum for T3 and T4 determination was collected 37 days after the start of treatment. Results are presented as the mean ± S.E.M. of four rats per treatment group, except for the tamoxifen plus estradiol benzoate treatment group where $n = 5$. ⋆, significantly different from the vehicle-treated group ($p < 0.05$).

A small change in the GH secretion pattern and a sustained reduction in the serum T3 level accompanied the reduction in weight gain. The relatively minor effects of tamoxifen treatment on plasma GH levels are in contrast with results of an earlier report in which tamoxifen inhibited GH secretion in intact female rats for 7 weeks after administration (Tannenbaum et al., 1992). We have no explanation for the discrepancy, but other studies have shown that tamoxifen either increases or has no effect on serum GH levels (DiPippo et al., 1995; Borski et al., 1996). The reduction in serum T3 levels in intact female rats observed in the present study 5 weeks after tamoxifen treatment represents a novel finding. The correlation between decreased serum T3 concentration and decreased body weight suggests that the effect of tamoxifen on weight gain may be T3- rather than GH-mediated. Studies conducted with ovariectomized-hypothyroid rats indicate that tamoxifen can inhibit the stimulatory effect of T3 on somatic growth (DiPippo and Powers, 1997; Fitts et al., 1998). The hormonal interplay between estradiol and T3 in regulating growth is complex and the mechanism by which tamoxifen reduces circulating T3 levels is not known.

Tamoxifen has been shown to act as an estrogen agonist or antagonist in rat uterus depending on the gonadal status of the animal. Tamoxifen mimics the activity of estrogen and increases uterine weight in immature and ovariectomized adult rats (Bowman et al., 1983; Wakeling et al., 1991) but decreases uterine weight in intact adult rats (Wakeling and Bowler, 1988). The sustained decrease in uterus weight by tamoxifen noted in the current study is suggestive of antiestrogenic activity. Treatment with ICI 182,780 for 14 days has also been shown to decrease uterine weight in intact adult rats (Wakeling et al., 1991). The reason for the lack of effect by ICI 182,780 on uterine weight in our study is not apparent, but a partial explanation may be that the half-life of ICI 182,780 is shorter than that of tamoxifen (10 h) or 4-hydroxytamoxifen (17 h) (Robinson et al., 1991). In this case, the suppressive effect of ICI 182,780 on uterine weight would have disappeared quickly, whereas the suppressive effect of tamoxifen would have persisted.

In summary, the present study demonstrates that 2-day treatment with tamoxifen at dosages as low as 0.5 mg/kg decreased body weight and serum T3 levels, and at a dosage of 20 mg/kg decreased peak plasma GH levels, hepatic CYP2A1 expression, and uterus and ovary weight for more than 5 weeks in intact adult female rats. The pattern of effects produced by tamoxifen did not resemble that produced by ICI 182,780 or estradiol benzoate but was similar to that of 4-hydroxytamoxifen, suggesting that this metabolite contributes to the activity of tamoxifen in vivo. The sustained effects of tamoxifen are likely to be mediated indirectly and cannot be explained by a single mechanism. The suppression of food intake and body weight caused by tamoxifen exhibits characteristics of a T3-dependent process. However, the precise neuroendocrine mechanisms responsible for effects of tamoxifen on hepatic CYP2A1 expression, T3 levels, and uterine weight remain to be elucidated.

Acknowledgments

We thank Dr. Paul E. Thomas (Rutgers The State University of New Jersey) for providing us with polyclonal antibody to CYP2A1 and Dr. Andrew Parkinson (University of Kansas Medical Center) for providing us with purified CYP2A1. We are grateful to Andreas Melhorn for his help in developing the HPLC assay for tamoxifen and its metabolites. We also thank Dr. Thomas K. H. Chang for his critical review and helpful suggestions during the preparation of the manuscript.

References

Anderson MD, Bandiera SM, Chang TKH, and Bellward GD (1998) Effect of androgen administration during puberty on hepatic CYP2C11, CYP3A and CYP2A1 expression in adult female rats. *Drug Metab Dispos* **26:**1031–1038.

Bandiera S and Dworschak C (1992) Effects of testosterone and estrogen on hepatic levels of cytochromes P450 2C7 and P450 2C11 in the rat. *Arch Biochem Biophys* **286:**286–295.

Biegel LB, Flaws JA, Hirschfield AN, O'Connor JC, Elliot GS, Ladics GS, Silbergeld EK, van Pelt CS, Hurtt ME, Cook JC, et al. (1998) 90-Day feeding and one-generation reproduction study in Crl:CD BR rats with 17β-estradiol. *Toxicol Sci* **44:**116–142.

Borski RJ, Tsai WDE, Mott-Friberg R, and Barkan AL (1996) Regulation of somatic growth and the somatotropic axis by gonadal steroids: primary effect on insulin-like growth factor I expression and secretion. *Endocrinology* **137:**3253–3259.

Bowman SP, Jones CA, Leake A, and Morris ID (1983) The biological activity of a single dose of tamoxifen in the adult ovariectomized rat. *Br J Pharmacol* **78:**617–622.

Chang TKH, Chan MMY, Holsmer SL, Bandiera SM, and Bellward GD (1996) Impact of tamoxifen on peripubertal androgen imprinting of rat hepatic cytochrome P450 2C11, cytochrome P450 3A2 and steroid 5α-reductase. *Biochem Pharmacol* **51:**357–368.

Dehal SS and Kupfer D (1999) Cytochrome P-450 3A and 2D6 catalyze *ortho* hydroxylation of 4-hydroxytamoxifen and 3-hydroxytamoxifen (droloxifene) yielding tamoxifen catechol: involvement of catechols in covalent binding to hepatic proteins. *Drug Metab Dispos* **27:**681–688.

DiPippo VA, Lindsay R, and Powers CA (1995) Estradiol and tamoxifen interactions with thyroid hormone in the ovariectomized-thyroidectomized rat. *Endocrinology* **136:**1020–1033.

DiPippo VA and Powers CA (1997) Tamoxifen and ICI 182,780 interactions with thyroid hormone in the ovariectomized-thyroidectomized rat. *J Pharmacol Exp Ther* **281:**142–148.

Edén S (1979) Age- and sex-related differences in episodic growth hormone secretion in the rat. *Endocrinology* **105:**555–560.

Fitts JM, Klein RM, and Powers CA (1998) Comparison of tamoxifen effects on the actions of triiodothyronine or growth hormone in the ovariectomized-hypothyroid rat. *J Pharmacol Exp Ther* **286:**392–402.

Fornander T, Rutqvist LE, Cedermark B, Glas U, Mattsson A, and Silfversward C (1989) Adjuvant tamoxifen in early breast cancer: occurrence of new primary tumors. *Lancet* **1:**117–120.

Greaves P, Goonetilleke R, Nunn G, Topham J, and Orton T (1993) Two-year carcinogenicity study of tamoxifen in Alderley Park Wistar-derived rats. *Cancer Res* **53:**3919–3924.

Hart JE (1990) Endocrine pathology of estrogens: species differences. *Pharmacol Ther* **47:**203–218.

Jordan VC (1976) Effect of tamoxifen (ICI 46,474) on initiation and growth of DMBA-induced rat mammary carcinomata. *Eur J Cancer* **12:**419–424.

Kawai M, Bandiera SM, Chang TKH, and Bellward GD (2000) Growth hormone regulation and developmental expression of rat hepatic CYP3A18, CYP3A9 and CYP3A2. *Biochem Pharmacol* **59:**1277–1287.

Kawai M, Bandiera SM, Chang TKH, Poulet FM, Vancutsem PM, and Bellward GD (1999) Modulation of hepatic CYP2A1, CYP2C11 and CYP3A9 expression in adult rats by neonatal administration of tamoxifen. *Drug Metab Dispos* **27:**1392–1389.

MacCallum J, Cummings J, Dixon JM, and Miller WR (1996) Solid-phase extraction and high-performance liquid chromatographic determination of tamoxifen and its major metabolites in plasma. *J Chromatogr B* **678:**317–323.

MacGregor JI and Jordan VC (1998) Basic guide to the mechanisms of antiestrogen action. *Pharmacol Rev* **50:**151–196.

Mani C, Gelboin HV, Park SS, Pierce R, Parkinson A, and Kupfer D (1993) Metabolism of the antimammary cancer antiestrogenic agent tamoxifen. I. Cytochrome P-450-catalyzed N-demethylation and 4-hydroxylation. *Drug Metab Dispos* **21:**645–656.

Potter GA, McCague R, and Jarman M (1994) A mechanistic hypothesis for DNA adduct formation by tamoxifen following hepatic oxidative metabolism. *Carcinogenesis* **15:**439–442.

Robertson DW, Katzenellenbogen JA, Long DJ, Rorke EA, and Katzenellenbogen BS (1982) Tamoxifen antiestrogens. A comparison of the activity, pharmacokinetics and metabolic activation of the *cis* and *trans* isomers of tamoxifen. *J Steroid Biochem* **16:**1–13.

Robinson SP, Langan-Fahey SM, Johnson DA, and Jordan VC (1991) Metabolites, pharmacodynamics and pharmacokinetics of tamoxifen in rats and mice compared to the breast cancer patient. *Drug Metab Dispos* **19:**36–43.

Smith LL, Brown K, Carthew P, Lim C-K, Martin EA, Styles J, and White INH (2000) Chemoprevention of breast cancer by tamoxifen: risks and opportunities. *Crit Rev Toxicol* **30:**571–594.

Tannenbaum GS, Gurd W, Lapinte M, and Pollak M (1992) Tamoxifen attenuates pulsatile growth hormone secretion: mediation in part by somatostatin. *Endocrinology* **130:**3395–3401.

Tartellin MF and Gorski RA (1971) Variations in food and water intake in the normal and acyclic female rat. *Physiol Behav* **7:**847–852.

Valette A, Verine A, Varesi L, and Boyer J (1980) Effects of ethynyl estradiol and progesterone on triglyceride metabolism in the female rat. *Endocrinology* **103:**1647–1653.

Wade GN and Gray JM (1979) Gonadal effects on food intake and adiposity: a metabolic hypothesis. *Physiol Behav* **22:**583–593.

Wade GN and Heller HW (1993) Tamoxifen mimics the effects of estradiol on food intake, body weight and body composition in rats. *Am J Physiol* **264:**R1392–R1398.

Wakeling AE and Bowler J (1988) Biology and mode of action of pure antiestrogens. *J Steroid Biochem* **30:**141–147.

Wakeling AE, Dukes M, and Bowler J (1991) A potent specific pure antiestrogen with clinical potential. *Cancer Res* **51:**3867–3873.

Waxman DJ and Chang TKH (1995) Hormonal regulation of liver cytochrome P450 enzymes, in *Cytochrome P450: Structure, Mechanism and Biochemistry*, 2nd ed (Ortiz de Montellano PR ed) pp 391–417, Plenum Press, New York.

Waxman DJ, Morrissey JJ, and LeBlanc GA (1989) Female-predominant rat hepatic P-450 forms j (IIE1) and 3 (IIA1) are under hormonal regulatory controls distinct from those of the sex-specific P-450 forms. *Endocrinology* **124:**2954–2966.

White INH, Davies A, Smith LL, Dawson S, and de Matteis F (1993) Induction of CYP2B1 and 3A1 and associated monooxygenase activities by tamoxifen and certain analogues in the livers of female rats and mice. *Biochem Pharmacol* **45:**21–30.

Wong A and Bandiera SM (1996) Inductive effect of Telazol® on hepatic expression of cytochrome P450 2B in rats. *Biochem Pharmacol* **52:**735–742.

Yamazoe Y, Ling X, Murayama N, Gong D, Nagata K, and Kato R (1990) Modulation of hepatic level of microsomal testosterone 7α-hydroxylase, P-450a (P450IIA), by thyroid hormone and growth hormone in rat liver. *J Biochem* **108:**599–603.

Address correspondence to: Dr. Stelvio Bandiera, Faculty of Pharmaceutical Sciences, University of British Columbia, 2146 East Mall, Vancouver, British Columbia, Canada V6T 1Z3. E-mail: bandiera@interchange.ubc.ca