Tamoxifen Alters Hepatic Cytochrome P450 Enzyme Expression and Circulating Growth Hormone Levels in Intact Male Rats

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Purpose. To investigate the effects of acute tamoxifen treatment on hepatic cytochrome P450 (CYP) expression and circulating thyroid and growth hormone (GH) levels in intact adult male rats.

Methods. Rats were injected subcutaneously with peanut oil (vehicle) or tamoxifen at a dosage of 20 or 200 mg/kg for 2 consecutive days. Blood for GH measurements was collected on day 34. Rats were sacrificed at 37 days after treatment, trunk blood was collected, and hepatic microsomes were prepared.

Results. Mean body weight of rats treated with tamoxifen at 200 mg/kg was decreased compared to vehicle-treated rats throughout the 5-week period after treatment. Hepatic CYP2A1-dependent testosterone 7α-hydroxylase activity and CYP2A1 protein content were increased, whereas CYP2C11-mediated testosterone 2α- and 16α-hydroxylase activities and CYP2C11 protein content were decreased significantly following tamoxifen administration. Peak plasma GH levels were 60% lower and nadir plasma GH levels were 30% higher in tamoxifen-treated relative to vehicle-treated rats. In contrast, serum triiodothyronine and thyroxine levels were not affected by tamoxifen treatment.

Conclusions. Hepatic CYP enzyme expression was altered and body weight was decreased in adult male rats 5 weeks after treatment with tamoxifen. This alteration corresponded to changes in plasma GH levels.

KEY WORDS: cytochrome P450; growth hormone; hormonal regulation; tamoxifen; thyroid hormones.

INTRODUCTION

Tamoxifen (Nolvadex, Zeneca Pharmaceuticals, Wilmington, DE) is a nonsteroidal partial antagonist of the estrogen receptor that is used widely in the treatment of all stages of breast cancer in both premenopausal and postmenopausal women (1,2). Clinical and animal studies indicate that chronic or short-term tamoxifen treatment can have diverse pharmacological actions including disruption of endocrine activity mediated by pituitary and thyroid hormones. For example, blood levels of follicle stimulating hormone, luteinizing hormone, prolactin, testosterone (3,4) and insulin-like growth factor-1 (5) were decreased, while triiodothyronine (T3), thyroxine (T4), thyroid-binding globulin, sex hormone binding globulin, and estradiol levels were increased (3,6,7) in postmenopausal breast cancer patients on tamoxifen therapy (10 mg twice daily). Tamoxifen administration at 10 mg twice daily for 4 days reduced plasma growth hormone (GH) concentrations in teenaged boys 3 to 4 days after treatment (8). Treatment of intact male and female rats with tamoxifen for 2 days was found to suppress GH secretion for up to seven weeks following administration of the drug (9). Tamoxifen also inhibited T3-dependent responses and increased mean plasma GH concentration in ovariectomized-hypothyroid female rats (10–12) and decreased serum testosterone concentration in adult male rats (13).

Thyroid hormones and the sexually dimorphic pattern of GH secretion play a major regulatory role in maintaining the sex- and age-dependent expression of hepatic drug- and steroid-metabolizing cytochrome P450 (CYP) enzymes such as CYP2A1, CYP2C11, and CYP2C12 in rat liver (14,15). Kawai *et al.* (16) reported that hepatic CYP2A1, CYP2C11, and CYP3A9 expression, testicular weight, and serum 17β-estradiol concentration were altered in adult rats as a result of neonatal tamoxifen administration. More recently, we demonstrated that treatment of adult female rats with tamoxifen for 2 days led to decreases in serum T3 concentration and hepatic CYP2A1 protein levels 5 weeks after drug administration (17).

In the present study, we set out to determine if acute treatment of sexually mature intact male rats with tamoxifen alters the expression of hormonally regulated hepatic CYP enzymes. Circulating GH and thyroid hormone levels were also measured to gain further insight into the mechanism by which tamoxifen modulates CYP enzyme expression.

MATERIALS AND METHODS

Animal Treatment

Adult male Long Evans rats, approximately 65–80 days of age (313–364 g), (Charles River Laboratories, Montreal, Quebec, Canada) were housed in pairs on corn-cob bedding in polycarbonate cages with free access to water and a commercial rat diet (Rodent Laboratory Diet, No. 5001, PMI Feeds Inc., Richmond, IN). Animal quarters were maintained at a temperature of 20–23°C and had a 12-h photoperiod. The research adhered to the principles and guidelines of the Canadian Council on Animal Care.

Rats were divided randomly into a control and a treatment group, with 6 to 9 animals in each group, in two separate experiments. After a 3-day acclimation period, rats were injected s.c. with tamoxifen (Sigma Chemical Co., St Louis, MO) in peanut oil at a dosage of 5 mg/rat (equivalent to 20 mg/kg) or 200 mg/kg on two consecutive days. A supraphysiologic dosage of 200 mg/kg tamoxifen was selected for testing to maximize potential effects observed with the lower tamoxifen dosage. The control group received the vehicle 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 a.m.) on day 34 from 3 rats treated with tamoxifen at a dosage of 200 mg/kg and from 3 control rats. 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)

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ABBREVIATIONS: CYP, cytochrome P450; IgG, immunoglobulin G; GH, growth hormone; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; T3, triiodothyronine; T4, thyroxine.

and placed on ice. Rats were killed by decapitation 37 days after the start of treatment and trunk blood was collected, placed on ice and allowed to clot. Livers were excised immediately, blotted dry, and weighed. Serum or plasma was separated from blood by centrifugation at 13,000 × *g* for 15 min at 4°C and stored immediately at −75°C until analysis. Hepatic microsomes were prepared from individual rats by differential ultracentrifugation (17). The final microsomal pellet was suspended in 0.25 M sucrose and aliquots of the suspension were stored at −75°C.

CYP Assays

Total protein concentrations were determined by using the method of Lowry *et al.* (18). Total CYP concentrations were determined by the sodium dithionite reduced carbon monoxide difference spectrum, using a molar extinction coefficient of 91 cm^{-1} mm^{-1} (19). Microsomal testosterone hydroxylase activity was determined as reported elsewhere (20). In brief, the reaction mixture contained 0.92 ml of 50 mM potassium phosphate buffer with 3 mM $MgCl_2$ at, pH 7.4, 50 μl of hepatic microsomes (at 6 nmol/ml), and 10 μl of 100 mM NADPH. The reaction was initiated by the addition of 20 μl of 12.5 mM testosterone and test tubes were incubated for 5 min at 37°C in a water bath with shaking. The reaction was stopped with 6 ml of dichloromethane per test tube, and 50 μl of 50 μM 11β-hydroxytestosterone was then added as the internal standard. Testosterone metabolites were extracted, dried under nitrogen, reconstituted in 200 ml of methanol, and quantified by HPLC analysis using calibration curves of authentic hydroxytestosterone standards.

SDS-PAGE and Immunoblot Assay

Hepatic microsomal CYP2A1, CYP2A2, CYP2B1, CYP2B2, CYP2C11, CYP2C12, and CYP3A enzymes were separated by SDS-PAGE and protein levels were quantified by immunoblot analysis as described previously (16,17,20). 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 IgG/ml, rabbit anti-CYP2B1 IgG at 2 μg IgG/ml, rabbit anti-CYP2C11 monospecific IgG at 15 μg IgG/ml, mouse anti-CYP2C12 monoclonal IgG at 2.5 μg IgG/ml, or rabbit anti-CYP3A1 IgG at 50 μg 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 or anti-mouse IgG (TAGO Immunologicals Inc., Burlingame, 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.

Rat CYP2A2 and CYP2C12, expressed in insect cell supersomes, were purchased from BD Biosciences (Bedford, MA). Purified rat CYP2A1 was obtained from Dr. A. Parkinson (XENOTECH LLC, Kansas City, KS City). CYP2B1, CYP2B2, CYP2C11, and CYP3A1 were purified as reported previously (16,17,20). Dr. P. E. Thomas (Rutgers - The State University of New Jersey, Piscataway, NJ) provided the anti-CYP2A1 IgG and Dr. E. T. Morgan (Emory University, Atlanta, GA) provided the anti-CYP2C12 IgG.

Hormone Assays

Plasma GH and serum T3 and T4 concentrations were measured using commercial enzyme immunoassay kits for rat GH (Amersham Biosciences, Baie d'Urfé, QC, Canada) and solid phase RIA kits for thyroid hormones (ICN Biomedicals, Costa Mesa, CA), as outlined (17).

Statistical Analysis

Mean values of the control and treatment group were compared using the unpaired *t* test. Differences with a p value < 0.05 were considered to be significantly different.

RESULTS

Body Weight

The effects of tamoxifen were investigated in intact adult male rats using a treatment regimen that was previously associated with prolonged antineoplastic activity (21) and altered hepatic CYP2A1 expression in adult female rats (17). Administration of tamoxifen once daily for 2 days at a dosage of 200 mg/kg resulted in a marked suppression of body weight in adult male rats (Fig. 1), with a mean final body weight that was approximately 20% less than that of the vehicle-treated group. Body weight was decreased during the first ten days after treatment, followed by attenuated weight gain, which persisted up to 37 days after treatment. In a separate experiment, tamoxifen administered at 20 mg/kg had a similar negative effect on body weight. The final body weight of rats that received tamoxifen at the lower dosage (20 mg/kg s.c. once daily for 2 days) was 26% lower than that of the correspond-

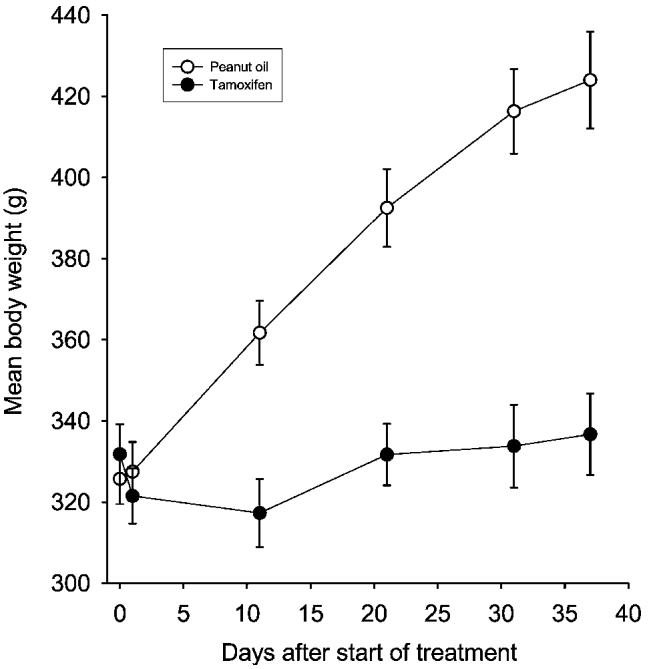

Fig. 1. Body weights of intact adult male rats at various times following treatment with tamoxifen at 200 mg/kg (●) or peanut oil (○). Body weights are presented as the mean ± SEM of 6 rats per group. Mean body weights of rats treated with tamoxifen were significantly different ($p < 0.05$) from those of control rats on days 11, 21, 31, and 37.

ing control group. Liver weight was unaffected by the tamoxifen treatment (data not shown).

Hepatic Microsomal CYP Activities and Content

The effect of tamoxifen on CYP expression was investigated by measuring CYP-mediated catalytic activities and protein levels in hepatic microsomes. Total CYP content, expressed per mg total protein, was not significantly different between vehicle-treated and tamoxifen-treated (20 or 200 mg/kg) rats (Table I). However, testosterone 7α-hydroxylase activity, which is a selective marker for CYP2A1, was increased by approximately 60% following treatment with tamoxifen at 20 or 200 mg/kg. In contrast, testosterone 2α- and 16α-hydroxylase activities, which serve as catalytic markers for CYP2C11 expression, were decreased by approximately 40% following treatment with tamoxifen at 200 mg/kg (Table I). The rates of formation of 6β- and 16β-hydroxytestosterone and androstenedione, which reflect the activities of CYP2B and CYP3A enzymes, were not affected by tamoxifen treatment. Testosterone 2β-hydroxylase, a CYP3A-catalyzed activity, was decreased following treatment with tamoxifen at 20 mg/kg.

CYP protein levels were measured in hepatic microsomes of tamoxifen-treated rats (200 mg/kg) by densitometric quantification of immunoblots probed with specific antibodies. Immunoblot analysis confirmed that tamoxifen treatment increased CYP2A1 and decreased hepatic CYP2C11 protein levels by approximately 30% in each case, relative to the control group (Fig. 2A). The hepatic level of CYP2A2, which is a male-specific enzyme like CYP2C11, was determined to be approximately 1 pmol/nmol total CYP and was not significantly different between tamoxifen- and vehicle-treated rats. CYP2C12, a female-specific enzyme, was not detectable in hepatic microsomes from either tamoxifen-treated or control male rats. Hepatic CYP2B1, CYP2B2, and CYP3A protein levels were not affected by tamoxifen treatment (data for CYP2B1 + CYP2B2 and CYP3A shown in Fig. 2B).

GH and Thyroid Hormone Levels

To determine if changes in body weight and hepatic CYP enzyme expression were associated with altered GH secretion by tamoxifen, plasma GH concentrations were determined for 3 vehicle-treated and 3 tamoxifen-treated (200 mg/kg) rats during an 8-h period 3 days before rats were killed (Fig. 3). Individual representative 8-h plasma GH profiles are presented in Fig. 3. The plasma GH profile of the tamoxifen-treated rat was characterized by fewer peaks and troughs compared to that of the vehicle-treated rat. Mean plasma GH levels were the same but peak plasma GH levels were approximately 60% lower ($p < 0.05$) and nadir plasma GH levels were approximately 30% higher ($p < 0.05$) in tamoxifen-treated rats compared to the control rats (Table II). Serum T3 and T4 concentrations measured 37 days after the start of treatment were not significantly different between vehicle-treated rats and rats treated with tamoxifen at 200 mg/kg (Fig. 4).

DISCUSSION

Tamoxifen is a selective estrogen receptor modulator that is capable of acting as a full estrogen agonist, partial agonist, or antagonist depending on the species and tissue (1). Previous studies have shown that brief or chronic exposure to tamoxifen during development produced long-lasting changes including altered expression of hepatic CYP enzymes in rats (13,16). The present study demonstrates that hepatic CYP2A1 and CYP2C11 expression and somatic growth were affected in sexually mature male rats following treatment with tamoxifen for 2 days.

The reduction in body weight associated with tamoxifen treatment of male rats (20–26% decrease relative to the vehicle-treated group) did not appear to be dosage-dependent. Comparable reductions in body weight (decrease of 12–18%) were reported recently for female rats treated for 2 days at dosages of 0.5 to 200 mg/kg (17). Decreases in body weight were also observed for intact male and female rats that were treated with tamoxifen daily for 2 years at dosages that ranged from 5 to 35 mg/kg/day (22). In that study, male rats exhibited greater weight gain suppression than female rats, an effect that was attributed to lower food consumption (22). Decreased food intake and somatic growth have been ascribed to the weak estrogenic activity of tamoxifen (23). For female rats that were exposed to tamoxifen using the same regimen as that used herein, food intake was reduced for only the first 2 weeks following treatment but body weight remained depressed for up to 5 weeks (17), suggesting that food intake alone is unlikely to account for the persistent suppression in weight gain noted previously (17) and in the current study.

We had suspected previously that T3 suppression played a role in suppressing somatic growth (17). In the present study, serum T3 levels were not affected by tamoxifen. In contrast, plasma GH peak levels were decreased and GH nadir levels were elevated five weeks after tamoxifen administration. Tannenbaum *et al.* (9) also found that peak GH levels were decreased following treatment with tamoxifen for 2 days, but they reported a more profound attenuation of GH secretion than was seen in this study. The reduced weight gain

Table I. Effect of Tamoxifen Treatment on Hepatic Microsomal CYP Content and Testosterone Hydroxylase Activities

Dosage (mg/kg)	Total CYP content (nmol/mg)*	Testosterone metabolite (pmol formed·min^{-1}·nmol^{-1} total CYP)						
		2α	2β	6β	7α	16α	16β	Androstenedione
0	1.28 ± 0.24	1580 ± 135	185 ± 48	1180 ± 178	154 ± 25	2450 ± 196	44 ± 5	3160 ± 390
20	1.26 ± 0.17	1256 ± 132	124 ± 20†	1180 ± 122	243 ± 46†	2020 ± 211	41 ± 2	2980 ± 193
0	1.07 ± 0.06	1540 ± 104	179 ± 9	1780 ± 168	238 ± 33	2650 ± 284	77 ± 15	1600 ± 183
200	0.95 ± 0.05	872 ± 84†	172 ± 22	1840 ± 212	387 ± 47†	1570 ± 150†	78 ± 8	1480 ± 108

* Total CYP content and enzyme activities are expressed as mean the ± SEM for 6–9 rats per treatment group.
† Significantly different ($p < 0.05$) from the corresponding vehicle-treated (0 mg/kg) group.

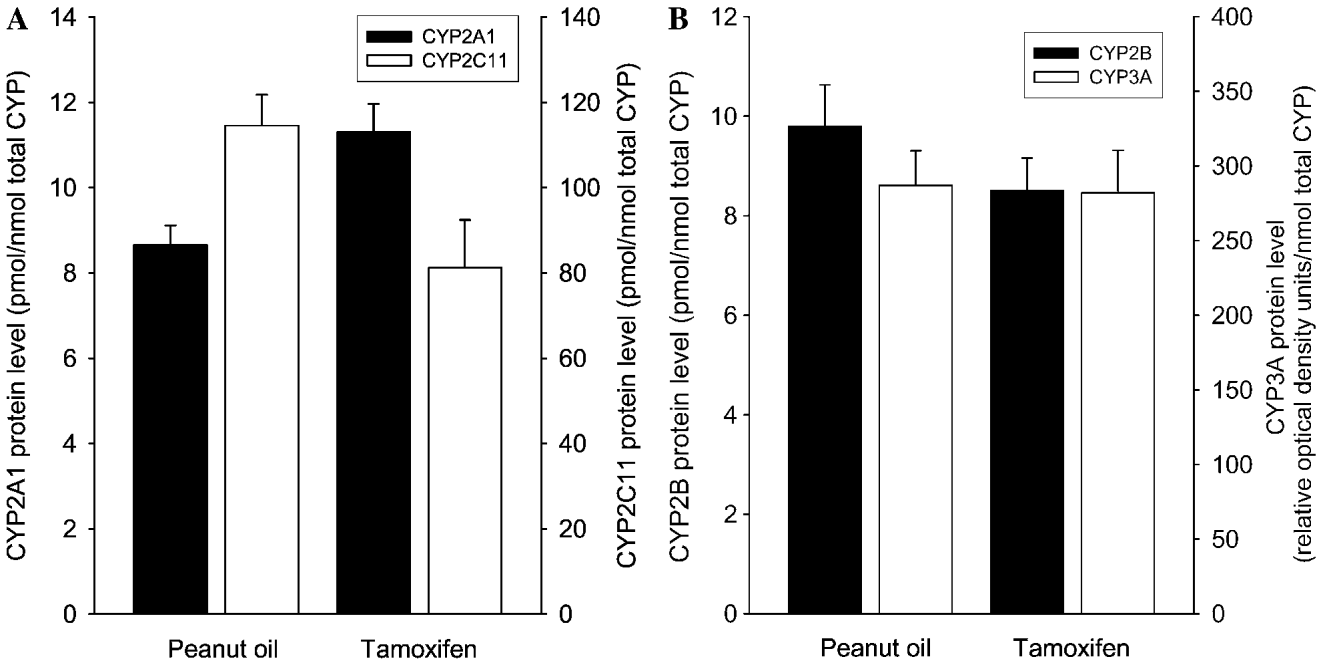

Fig. 2. Hepatic microsomal levels of CYP proteins in intact adult male rats following treatment with tamoxifen at 200 mg/kg. Hepatic microsomes were prepared 37 days after the start of treatment and CYP protein levels were measured by densitometric analysis of immunoblots probed with polyclonal antibodies as described in "Materials and Methods." (A) CYP2A1 (left vertical axis and solid bars) and CYP2C11 (right vertical axis and open bars), (B) CYP2B (left vertical axis and solid bars) and CYP3A (right vertical axis and open bars). The CYP2B1 and CYP2B2 bands were quantified individually by densitometry and the data for both are presented. Values for CYP3A are expressed as relative optical density units/nmol total CYP because anti-rat CYP3A1 IgG cross-reacts with CYP3A2 and may also recognize other CYP3A enzymes that are unresolved from CYP3A1 on SDS-PAGE gels. Results are presented as the mean ± SEM of 6 rats per treatment group. *Significantly different from the vehicle-treated group ($p < 0.05$).

observed in our study could be explained by changes in plasma GH levels because decreased GH pulse height is associated with decreased body weight in rats (24,25).

The increase in hepatic CYP2A1 expression and decrease in CYP2C11 expression noted in the present study are consistent with results from Kawai *et al.* (16), in which hepatic CYP2A1 protein content was increased and CYP2C11 protein content was decreased in adult male rats that had been treated with tamoxifen during the neonatal period. In contrast, administration of tamoxifen to intact adult female rats, at dosages of 20 to 200 mg/kg, decreased CYP2A1 expression but had no effect on CYP2C11 expression (17). The mechanism by which tamoxifen alters CYP enzyme expression is unclear. Experimental data from our laboratory indicates that direct effects of tamoxifen on CYP expression are unlikely because neither tamoxifen nor 4-hydroxytamoxifen was detectable in plasma of adult female rats by 24 days after treatment with tamoxifen at a dosage of 50 mg/kg (17). Although it is possible that tamoxifen might be detected following treatment with the higher dosage used in the present study, we did not observe induction of CYP2B or CYP3A enzymes, which has been reported to be a direct effect of tamoxifen on CYP enzymes (26). In addition, tamoxifen does not appear to be acting as an estrogen receptor agonist in the liver because

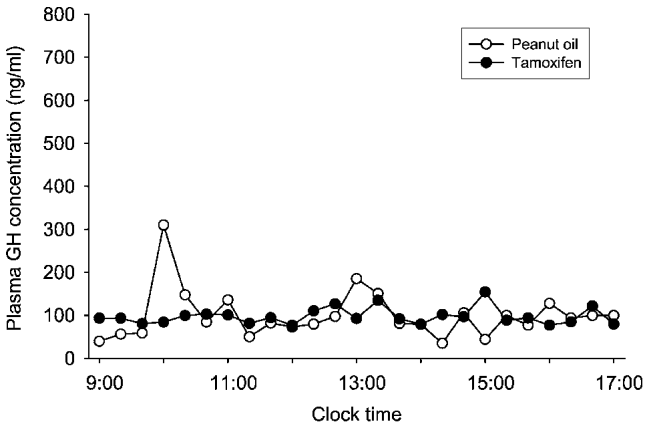

Fig. 3. Representative 8-h plasma GH concentrations following treatment with tamoxifen at 200 mg/kg (●) or peanut oil (○). Intact adult male rats were treated once daily for 2 consecutive days and blood samples (250 μl) were taken every 20 min for 8 h at 34 days after the start of treatment. Data from one rat in each treatment group are shown in the figure.

Table II. Mean, Nadir, and Peak Plasma GH Concentrations Following Tamoxifen Treatment

Plasma GH concentrations (ng/ml)	Treatment	
	Peanut oil	Tamoxifen
Mean GH concentration	123 ± 14	124 ± 16
Nadir GH concentration	64 ± 13	93 ± 10*
Peak GH concentration	451 ± 71	178 ± 23*

Intact adult male rats were treated once daily for 2 consecutive days with tamoxifen at 200 mg/kg or peanut oil and blood samples (250 μl) were taken every 20 min for 8 h at 34 days after the start of treatment from 3 rats per treatment group.
* Significantly different from the vehicle-treated group ($p < 0.05$).

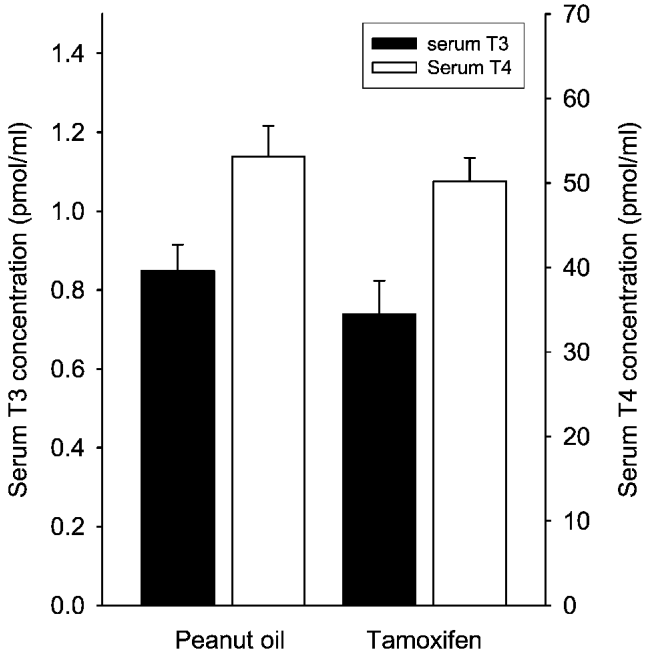

Fig. 4. Serum T3 (A) and T4 (B) concentrations of intact adult male rats following treatment with tamoxifen at 200 mg/kg. Trunk blood for T3 and T4 determinations was collected 37 days after the start of treatment. Results are presented as the mean ± SEM of 6 rats per treatment group.

treatment of intact male rats with estradiol benzoate did not increase testosterone 7α-hydroxylase activity, regardless of the dosage used (27).

Numerous studies have shown that hepatic expression of CYP2A1 and CYP2C11 is developmentally regulated and under the influence of gonadal steroids and the sexually dimorphic pattern of GH secretion. Gonadectomy, which reduces steroid hormone levels and alters GH secretion patterns, leads to increased CYP2A1 and decreased CYP2C11 expression in male rats and decreased CYP2A1 expression in female rats (28–30). Similar changes in CYP2A1 and CYP2C11 expression are produced in male rats by hypophysectomy (28,29). Moreover, alteration of the male specific GH secretion pattern by continuous infusion of exogenous GH, which elevates GH plasma nadir levels and suppresses GH peak levels, was found to induce CYP2A1 (28) and suppress CYP2C11 (29) expression in intact rats. Taken together, these studies indicate that CYP2A1 and CYP2C11 are sensitive to changes in the GH concentration profile. The reduction of peak plasma GH levels and the elevation of nadir GH levels observed after tamoxifen treatment could have had a partial feminizing effect on the sexually dimorphic expression of hepatic CYP2A1 and CYP2C11 levels in intact male rats. The absence of an effect on CYP2A2 and CYP2C12 expression, however, suggests that the feminization effect, if it occurred, was not large.

In summary, results from the present and recent studies establish that tamoxifen alters the hepatic CYP2A1 and CYP2C11 enzyme profile in male rats and hepatic CYP2A1 expression in female rats, regardless of whether the drug is administered during the neonatal or adult period. Moreover, the effect of a relatively brief exposure to tamoxifen is measurable for several weeks after treatment. The mechanism by which tamoxifen alters hepatic CYP expression remains unclear but the results are suggestive of an indirect hormonal effect. We speculate that different mediators are involved in male and female rats. In male rats, alterations of CYP enzyme expression are consistent with the conclusion that a reduction in peak plasma GH levels and an elevation in nadir plasma GH levels mediated the effects of tamoxifen. The relevance of our findings in relation to the therapeutic use of tamoxifen is unknown.

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REFERENCES

1. V. C. Jordan. Antiestrogens and selective estrogen receptor modulators as multifunctional medicines. 2. Clinical considerations and new agents. *J. Med. Chem.* **46**:1081–1111 (2003).
2. B. Fisher, J. P. Costantino, D. L. Wickerham, C. K. Redmond, M. Kavanah, W. M. Cronin, V. Vogel, A. Robidoux, N. Dimitrov, J. Atkins, M. Daly, S. Wieand, E. Tan-Chiu, L. Ford, and N. Wolmark. Tamoxifen for prevention of breast cancer: Report of the national surgical adjuvant breast and bowl project P-1 study. *J. Natl. Cancer Inst.* **90**:1371–1388 (1998).
3. K. Kostoglou-Athanassiou. Ntalles, J. Gogas, C. Markopoulos, V. Alevizou-Terzaki, P. Athanassiou, E. Georgiou, and C. Proukakis. Sex hormones in postmenopausal women with breast cancer on tamoxifen. *Horm. Res.* **47**:116–120 (1995).
4. P. E. Lønning, D. C. Johannessen, E. A. Lien, D. Ekse, T. Fotsis, and H. Adlercreutz. Influence of tamoxifen on sex hormones, gonadotrophins and sex hormone binding globulin in postmenopausal breast cancer patients. *J. Steroid Biochem. Mol. Biol.* **52**: 491–496 (1995).
5. M. N. Pollak, H. T. Huynh, and S. P. LeFebvre. Tamoxifen reduces serum insulin-like growth factor I (IGF-I). *Breast Cancer Res. Treat.* **22**:91–100 (1992).
6. C. C. Mamby, R. R. Love, and K. E. Lee. Thyroid function test changes with adjuvant tamoxifen therapy in postmenopausal women with breast cancer. *J. Clin. Oncol.* **13**:854–857 (1995).
7. J. Cuzick, D. Allen, M. Baum, J. Barrett, G. Clark, V. Kakkar, E. Melissari, C. Moniz, J. Moore, V. Parsons, K. Pemberton, P. Pitt, W. Richmond, J. Houghton, and D. Riley. Long term effects of tamoxifen. *Eur. J. Cancer* **29A**:15–21 (1992).
8. D. L. Metzger and J. R. Kerrigan. Estrogen receptor blockade with tamoxifen diminishes growth hormone secretion in boys: Evidence for a stimulatory role of endogenous estrogens during male adolescence. *J. Clin. Endocrinol. Metab.* **79**:513–518 (1994).
9. G. S. Tannenbaum, W. Gurd, M. Lapinte, and M. Pollak. Tamoxifen attenuates pulsatile growth hormone secretion: mediation in part by somatostatin. *Endocrinology* **130**:3395–3401 (1992).
10. V. A. DiPippo, R. Lindsay, and C. A. Powers. Estradiol and tamoxifen interactions with thyroid hormone in the ovariectomized-thyroidectomized rat. *Endocrinology* **136**:1020–1033 (1995).
11. V. A. DiPippo and C. A. Powers. Tamoxifen and ICI 182,780 interactions with thyroid hormone in the ovariectomized-thyroidectomized rat. *J. Pharmacol. Exp. Ther.* **281**:142–148 (1997).
12. J. M. Fitts, R. M. Klein, and C. A. Powers. Comparison of tamoxifen effects on the actions of triiodothyronine or growth hormone

in the ovariectomized-hypothyroid rat. *J. Pharmacol. Exp. Ther.* **286**:392–402 (1998).
13. A. Bartke, M. Mason, S. Dalterio, and F. Bex. Effects of tamoxifen on plasma concentrations of testosterone and gonadotrophins in the male rat. *J. Endocrinol.* **79**:239–240 (1978).
14. Y. Yamazoe, X. Ling, N. Murayama, D. Gong, K. Nagata, and R. Kato. 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 (1990).
15. D. J. Waxman and T. K. H. Chang. Hormonal regulation of liver cytochrome P450 enzymes. In: Ortiz de Montellano PR (ed.), *Cytochrome P450: Structure, Mechanism, and Biochemistry*, 2nd edition, Plenum Press, New York, 1995, pp. 391–417.
16. M. Kawai, S. M. Bandiera, T. K. H. Chang, F. M. Poulet, P. M. Vancutsem, and G. D. Bellward. Modulation of hepatic CYP2A1, CYP2C11, and CYP3A9 expression in adult rats by neonatal administration of tamoxifen. *Drug Metab. Disp.* **27**:1392–1399 (1999).
17. L. M. Ickenstein and S. M. Bandiera. Persistent suppression of hepatic CYP2A1 expression and serum T3 levels by tamoxifen in intact female rats: Dose-response analysis and comparison with 4-hydroxytamoxifen, ICI 182,780, and 17β-estradiol-3-benzoate. *J. Pharmacol. Exp. Ther.* **302**:584–593 (2002).
18. O. H. Lowry, N. J. Risebrough, A. C. Farr, and R. J. Randall. Protein measurement with the folin reagent. *J. Biol. Chem.* **193**: 265–275 (1951).
19. T. Omura and R. Sato. The carbon monoxide binding pigment of liver microsomes II. Solubilization and purification and properties. *J. Biol. Chem.* **239**:2379–2385 (1964).
20. M. D. Anderson, S. M. Bandiera, T. K. H. Chang, and G. D. Bellward. Effect of androgen administration during puberty on hepatic CYP2C11, CYP3A, and CYP2A1 expression in adult female rats. *Drug Metab. Disp.* **26**:1031–1038 (1998).
21. V. C. Jordan. Effect of tamoxifen (ICI 46,474) on initiation and growth of DMBA-induced rat mammary carcinomata. *Eur. J. Cancer* **12**:419–424 (1976).
22. P. Greaves, G. Goonetilleke, G. Nunn, J. Topham, and T. Orton. Two-year carcinogenicity study of tamoxifen in Alderly Park Wistar-derived rats. *Cancer Res.* **53**:3919–3924 (1993).
23. G. N. Wade and H. W. Heller. Tamoxifen mimics the effect of estradiol on food intake, body weight and body composition in rats. *Am. J. Physiol.* **264**:R1392–R1398 (1993).
24. W. B. Wehrenberg. The role of growth hormone-releasing factor and somatostatin on somatic growth in rats. *Endocrinology* **118**: 489–494 (1986).
25. M. D. Lumpkin, S. E. Mulroney, and A. Haramati. Inhibition of pulsatile growth hormone (GH) secretion and somatic growth in immature rats with a synthetic GH-releasing factor antagonist. *Endocrinology* **124**:1154–1159 (1989).
26. I. N. H. White, A. Davies, L. L. Smith, S. Dawson, and F. De-Matteis. 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 (1993).
27. O. Putz, C. B. Schwartz, G. A. LeBlanc, R. L. Cooper, and G. S. Prins. Neonatal low- and high-dose exposure to estradiol benzoate in the male rat: II. Effects on male puberty and the reproductive tract. *Biol. Reprod.* **65**:1506–1517 (2001).
28. D. J. Waxman, J. J. Morrissey, and G. A. LeBlanc. Female predominant rat hepatic P-450 forms j (IIEI) and 3 (IIAI) are under hormonal regulatory controls distinct from those of the sex-specific P-450 forms. *Endocrinology* **124**:2954–2966 (1989).
29. E. T. Morgan, C. MacGeoch, and J.-Å. Gustafsson. Hormonal and developmental regulation of expression of the hepatic microsomal steroid 16α-hydroxylase cytochrome P-450 apoprotein in the rat. *J. Biol. Chem.* **260**:11895–11898 (1985).
30. S. M. Bandiera and C. Dworschak. Effects of testosterone and estrogen on hepatic levels of cytochromes P450 2C7 and P450 2C11 in the rat. *Arch. Biochem. Biophys.* **286**:286–295 (1992).