Comparison of Tamoxifen Effects on the Actions of Triiodothyronine or Growth Hormone in the Ovariectomized-Hypothyroid Rat

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Accepted for publication March 16, 1998 This paper is available online at http://www.jpet.org

ABSTRACT

Recent studies have suggested that a subset of estrogen responses arise via modulation of triiodothyronine (T3) actions, and depend on T3 for expression: other estrogen responses are not T3-dependent. Moreover, tamoxifen acts as a full estrogen agonist in T3-dependent responses but behaves as an antiestrogen in T3-independent responses. T3 directly induces a variety of metabolic enzymes and proteins, and also induces rat growth hormone (GH). Thus, some T3-dependent tamoxifen effects might reflect modulation of GH rather than T3 actions. To address this issue, tamoxifen effects on somatotropic and metabolic actions of T3 and GH were compared in ovariectomized rats with methimazole-induced hypothyroidism. Rats were given T3 (10 μg/kg/day) or ovine GH (2 mg/kg/day) with or without tamoxifen (0.5 mg/kg/day) for 30 days. GH was poorly effective in producing a sustained increase in somatic growth in hypothyroid rats compared to T3; nonetheless, GH effects to increase food intake or body temperature. T3 alone increased body weight, tibia length and serum insulin-like growth factor I; T3 increased body temperature, insulin-like growth factor I levels and all measures of somatic growth and, unlike GH, increased food intake and tended to decrease tibia bone mineral density. Tamoxifen inhibited the somatotropic actions of T3 (including increases in insulin-like growth factor I levels), and produced significant increases in tibia bone mineral density only in T3-treated rats. Tamoxifen had no effect on T3 actions to increase food intake or body temperature. T3 alone increased fat mass and exhibited a tendency to decrease serum triglycerides: tamoxifen had no effect on these parameters in the absence of T3. However, coadministration of tamoxifen with T3 produced a marked decrease in fat mass and increased serum triglycerides. GH had no effect on serum triglycerides in either the presence or absence of tamoxifen. Serum glucose levels appeared normal in all groups. The data indicate that multiple tamoxifen effects on growth and metabolism may reflect modulation of T3 rather than GH actions.

T3 and estrogens activate selective members of a superfamily of nuclear receptors which act as ligand-regulated transcription factors (Carson-Jurica et al., 1990; Mangelsdorf et al., 1995). Receptors for E2, but not other steroid hormones, bind target response elements with two consensus AGGTCA motifs in a palindromic array with a 3-bp spacer: AGGTCA motifs in other orientations serve as targets for T3, retinoid and certain other receptors (Truss and Beato, 1993; Glass, 1994). Structural and functional similarities of ERs and TRs may enable them to modulate each other’s functions. Indeed, TRs bind certain EREs yet do not activate transcription (see Glass, 1994), and widely spaced, direct-repeat AGGTCA motifs may yield promiscuous sites for ER or TR binding and functional interplay (Kato et al., 1995). The failure of TRs to activate transcription when bound to EREs probably reflects the influence of response element structure on receptor conformation and interactions with coregulator proteins involved in transcriptional regulation (Kurokawa et al., 1995). Thus, ER or TR binding to DNA at some targets may depend on the modulation of other receptors rather than direct transcriptional effects. Evidence consistent with such interplay has been reported in studies using cells transfected with reporter gene and receptor constructs. TRs have been found to inhibit ER-mediated transactivation (Glass et al., 1988; Graupner et al., 1991; Segars et al., 1993; Zhu et al., 1996), and ER has been reported to inhibit TR actions (Adan and Burbach, 1992; Yarwood et al., 1993). In addition, ERs and TRs can activate and inhibit, respectively, transcription of target genes containing AP-1 elements by mechanisms independent of DNA binding (Desbois et al., 1991; Zhang et al., 1991; Segars et al., 1993; Webb et al., 1995; Paech et al., 1995).

Received for publication August 19, 1997.

1 This work was supported in part by Grant 94–404 from the American Heart Association, New York State Affiliate.

ABBREVIATIONS: BMD, bone mineral density; E2, 17β-estradiol; ER, estrogen receptor; GH, growth hormone; IGF-I, insulin-like growth factor I; T3, 3,3',5-triiodo-L-thyronine; T4, L-thyroxine; TR, thyroid hormone receptor; ERE, estrogen response elements.
ER-TR interplay offers a new physiological perspective for understanding how E2 may alter biological systems. ER modulation of TR function might relate to numerous metabolic actions of E2 because T3 has major effects on most of the metabolic systems affected by E2 (see DiPippo et al., 1995; DiPippo and Powers, 1997; Dellovade et al., 1995). To address this issue, two criteria were developed for identifying E2 responses that might arise from ER-TR interplay in vivo: 1) the E2 response should require T3 for expression (i.e., there must be a T3 response to be modulated) and 2) tamoxifen (an antiestrogen) should fully mimic E2 responses. This criteria was based on tamoxifen’s ability to fully mimic E2 in transforming ER to its DNA-binding form in vivo (Clark et al., 1973; Katzenellenbogen et al., 1979; Reese and Katzenellenbogen, 1992). Tamoxifen may thus fully mimic E2 in responses where induction of ER binding to DNA is sufficient to modulate TR function (see DiPippo and Powers, 1997). To identify physiological E2 responses arising from ER-TR interplay, E2 and tamoxifen effects on T3 actions were studied in ovariectomized-thyroidectomized rats. The model sought to detect pharmacodynamic interplay between T3 and E2 or tamoxifen while minimizing pharmacokinetic interactions that may confound interpretation. The studies showed that a subset of E2 responses require T3 for expression (T3-dependent responses). Moreover, tamoxifen behaved as a full E2 agonist in such responses, although simultaneously behaving as an antiestrogen in T3-independent responses (DiPippo and Powers, 1991; DiPippo et al., 1995; DiPippo and Powers, 1997). T3-dependent estrogen responses include inhibition of T3 effects to induce pituitary GH and somatic growth, inhibition of T3 induction of hepatic malic enzyme, effects to elevate serum triglycerides and effects to inhibit T3-evoked decreases in bone mineralization. T3-independent E2 responses include induction of prolactin, pituitary kallikrein and uterine growth, suppression of LH secretion, and GH induction in the absence of T3. Effects of T3 to induce prolactin or suppress TSH release are not affected by E2 or tamoxifen, and indicate that E2 or tamoxifen modulation of T3 effects is response selective.

Although E2 and tamoxifen inhibit T3 induction of pituitary GH (up to 50%), they either increase or do not affect serum GH levels (Carlsson et al., 1987; DiPippo et al., 1995; Borski et al., 1996; DiPippo and Powers, 1997). Indeed, E2 or tamoxifen failed to stimulate growth even while increasing pituitary and serum GH in the absence of T3 (DiPippo et al., 1995). This suggests that E2 and tamoxifen may directly inhibit GH effects. However, T3 is required for GH to evoke maximal growth effects (Simpson et al., 1950; Asling et al., 1954; deGroot, 1963; Thorngren and Hansson, 1973; Lewinson et al., 1994). Thus, physiological GH levels evoked by E2 or tamoxifen may be inadequate to stimulate growth without T3, and E2 and tamoxifen may inhibit T3-induced growth by blocking T3 effects to potentiate GH actions.

In theory, hypophysectomized rats given T3 or GH alone or together could be used to analyze questions relating to estrogen modulation of GH vs. T3 actions. However, in our hands almost 50% of hypophysectomized rats die from myxedema coma when given antithyroid drugs such as methimazole to eliminate basal T3 synthesis (Fitts and Powers, in preparation). Many hypophysectomized rats appear to maintain significant T3 production which is critical for homeostasis and animal viability.

In an alternate approach to address the role of GH in T3 actions, this study compared tamoxifen effects on the actions of T3 or ovine GH in ovariectomized rats with hypothyroidism induced by methimazole. In this model, hypothyroidism obliterates pituitary GH production. T3 both induces GH and can activate other metabolic or somatotropic effects independent of GH: ovine GH treatment allows effects due to GH alone to be assessed. Thus, tamoxifen effects to inhibit the somatotropic and metabolic effects of T3, but not GH, would indicate that tamoxifen may be primarily targeting responses dependent on T3. Conversely, tamoxifen effects to inhibit ovine GH actions would indicate direct inhibition of GH effects independent of T3. The results suggest that tamoxifen effects on growth and metabolism primarily reflect modulation of T3 actions. The study also further characterized the profile of somatotropic and metabolic effects of T3 which are modulated by tamoxifen.

Materials and Methods

Animals. All procedures were approved by the institutional Animal Care and Use Committee following guidelines approved by the National Institutes of Health. Thirty-six female Sprague-Dawley rats (175–200 g, Taconic Farms, Germantown, PA) were continuously given 0.03% methimazole in drinking water to induce hypothyroidism. Cessation of growth (less than 0.5 g weight gain per week) provided a functional index of hypothyroidism. Rats were ovariectomized 3 wk after the start of methimazole as previously described (DiPippo et al., 1995). Two rats did not survive ovariectomy due to impaired hemostasis and/or wound healing secondary to hypothyroidism. Thus, 34 rats completed the study and were analyzed for hormone and drug effects.

Experimental design and drug treatments. T3 and tamoxifen treatments were begun 1 wk after ovariectomy (4 wk after start of methimazole). Rats were divided into six groups: 1) vehicle control (n = 5); 2) tamoxifen alone (n = 5); 3) GH alone (n = 6); 4) T3 alone (n = 6); 5) GH plus tamoxifen (n = 6) and 6) T3 plus tamoxifen (n = 6). A physiological replacement dose of T3 (sodium salt) (10 μg/kg; Sigma Chemical Co., St. Louis, MO) or its vehicle was administered ip every 24 hr in 0.9% NaCl containing 5 mM NaOH; other animals received vehicle. Dose-response studies have shown that such T3 doses restore normal pituitary GH content in thyroidectomized rats, and evoke maximal rates of weight gain (DiPippo et al., 1995). Use of T3 rather than T4 lessens the possibility that drug-evoked changes in transthyretin and T4-binding globulin (serum T4 binding proteins) may alter thyroid hormone pharmacokinetics and actions since T3 binds with 10-fold lower affinity than T4 to these serum proteins. Thus, T3 rapidly distributes to tissue compartments following administration whereas T4 remains concentrated in the vascular compartment for prolonged periods (Oppenheimer et al., 1970). Use of T3 also avoids the possibility of drug effects on T4 deiodination to T3.

Trans-tamoxifen (free base) (0.5 mg/kg; Sigma) was given s.c. every 24 hr in sesame oil containing 1% benzyl alcohol; control animals received vehicle. Dose-response studies in ovariectomized and ovariectomized-thyroidectomized rats have shown that 0.2 mg/kg tamoxifen produces near maximal estrogen agonist effects, as well as maximal antagonist effects on moderate doses of estradiol benzoate or T3 (Powers et al., 1989; DiPippo et al., 1995).

Ovine GH (NIDDK-oGH-14) was dissolved in a small amount of 10 mM NaOH, immediately diluted 10-fold in 100 mM Tris-HCl (pH 7.8) and then adjusted to give a concentration of 0.5 mg/ml in 20 mM Tris-HCl, pH 7.8, with 0.9% NaCl and 20 μg/ml leupeptin. The GH
solution was then sterile-filtered using a 0.22-μm syringe filter, and sterile 2-ml aliquots stored at −20°C until use. Rats received a total daily GH dose of 2 mg/kg divided into two equal doses given in the morning and late afternoon by i.p. injection. Control animals received vehicle. Drug and hormone treatments were given for 30 days (4% of rat life span) with five to six rats per group.

Rats were housed in plastic cages with sawdust bedding in animal quarters maintained at 24 to 25°C (76–78°F) to minimize the risk of hypothermia and debilitation due to thyroid deficiency. Body weight was measured twice to three times weekly using a digital balance. Food intakes and body temperatures were also measured periodically to assess T3 and tamoxifen effects on these parameters. Individual food intakes were not directly measured because rats were gang-caged in treatment groups to avoid the development of severe hypothermia due to hypothyroidism. Therefore, total food intake per group was measured on three different occasions, and the average food intake per rat calculated. Body temperature was measured using a digital thermometer with a rectal thermister probe.

**Tissue processing.** Rats were killed with 100 mg/kg sodium pentobarbital (i.p.) 18 to 24 hr after the last injection. Blood samples were obtained for serum IGF-I, glucose and triglyceride determinations within 3 to 5 min after pentobarbital injection, and tissues collected as previously described (DiPippo et al., 1995). Uteri were dissected in situ, drained of luminal fluid, and dried for 48 hr at room temperature before weighing as an index of tamoxifen effects in a T3-independent E2 response. Parametrial fat pads were dissected and wet weights obtained as an index of changes in fat mass in the rats. The left kidney was dissected, dried 48 hr and weighed to provide an index of changes in lean body mass. The right tibias were stripped of all muscle and connective tissue, and stored in 70% ethanol at 5°C until measurement of tibia length with calipers and analysis of bone mineral density.

**Biochemical analyses.** Serum glucose levels were measured using a colorimetric kit from Stanbio Laboratories (San Antonio, TX). Serum IGF-I levels were measured by radioimmunoassay with a kit from Nichols Institute Diagnostics (San Juan Capistrano, CA) using synthetic human IGF-I as a standard after acid-ethanol treatment of serum to denature IGF-binding proteins. Total serum triglyceride and BMD analysis. BMD scans of entire excised tibias in 70% ethanol were prepared by dual-energy x-ray absorptiometry using a QDR-1000 (Hologic, Waltham, MA) with a 0.025" collimator. High-resolution image analysis software (Hologic) was used to calculate BMD (g/cm²) in two subregions of the rat tibia as previously described (Shen et al., 1993; DiPippo et al., 1995). Subregions analyzed included the upper 25% of tibia length (proximal tibia) containing the cancellous bone-enriched proximal metaphysis and the middle 50% of the tibia (diaphysis) composed almost exclusively of cortical bone.

**Statistics.** Data were analyzed by one-analysis of variance followed by Duncan’s new multiple range test. Where appropriate, data were log transformed to equalize variances.

**Results**

**Effect of T3 and GH on tamoxifen actions to increase uterine dry weight.** Induction of uterine weight is a widely used bioassay for characterizing antiestrogens. Tamoxifen exhibits about 30% of the efficacy of E2 in uterine weight induction (Powers et al., 1989; DiPippo et al., 1995), and uterine growth has been identified as a T3-independent E2 response (DiPippo et al., 1995; DiPippo and Powers, 1997). As shown in figure 1, T3 alone had no effect on uterine weight, and tamoxifen produced equivalent increases in either the presence or absence of T3. GH alone tended to decrease uterine weight (-17%) and significantly potentiated tamoxifen-evoked increases (+20%). Neither T3 nor GH were required for tamoxifen to bind ER and evoke uterine growth.

The data also indicate that T3 is unlikely to markedly alter tamoxifen elimination to produce differential responses in the presence of T3. **Effect of tamoxifen on T3 and GH effects to increase weight gain.** Figure 2 shows the changes in body weight of rats treated with GH or T3 in the presence or absence of tamoxifen. Rats treated with either vehicle or tamoxifen alone exhibited weight loss during the experiment, and tamoxifen-treated rats lost significantly more weight (3–7 g) than vehicle-treated controls (P < .05; analysis of variance). In contrast, GH or T3 alone significantly increased weight during the experiment. GH and T3-evoked weight gain appeared equivalent during the first 16 days of treatment; however, by 21 days T3-evoked growth exceeded that of GH, and weights of GH-treated rats declined after day 21 whereas T3 continued to stimulate weight gain. The inability of GH to continuously increase body weight has also been observed with human GH in hypophysectomized female rats: a decline in the growth response was observed after 12 to 18 days (Groesbeck and Parlow, 1987; Fielder et al., 1996). In agreement with previous studies, tamoxifen markedly inhibited T3-evoked weight gain (fig. 2). Tamoxifen also appeared to inhibit GH-evoked weight increases during the first 16 days of treatment, but was without effect on day 21 and appeared to prevent decreases in body weight seen during the final 9 days of GH-treatment.

In figure 2, weight gain was calculated from starting weight on day 1 of the experiment. However, because tamoxifen alone significantly decreased weight relative to vehicle controls, weight gain evoked by T3 and GH was also plotted after adjustment for changes due to treatment with vehicle or tamoxifen alone (fig. 3). For example, on day 7, rats treated with vehicle alone or tamoxifen alone had lost an average of 5.4 and 9.4 g, respectively, relative to their starting weights. Thus, 5.4 and 9.4 g were added to the corresponding weight gains of GH- or T3-treated rats on day 7 to compensate for
these treatment effects and accurately calculate GH- and T3-evoked weight gain. As shown in figure 3, tamoxifen continued to display strong inhibition of T3 effects to increase body weight after adjustment. However, tamoxifen did not significantly inhibit GH effects to increase weight. Indeed, tamoxifen protected against weight loss during the last 9 days of GH treatment. Thus, it was concluded that tamoxifen inhibits T3 effects to increase weight, but does not inhibit GH-evoked weight gain.

**Tamoxifen effects on tibia length, serum IGF-I levels and kidney mass.** At the end of the study, the length of the right tibia was determined to provide an index of longitudinal growth during the experiment. As shown in figure 4, both GH and T3 increased tibia length, but T3-evoked increases (2.34 mm) were over three times that produced by GH (0.70 mm). Tamoxifen alone had no effect on tibia length and inhibited T3-evoked tibia growth but did not inhibit GH effects to increase tibia length. This matches the differential sensitivity of T3 and GH effects to increase body weight.

Previous studies have clearly shown that T3 actions to increase serum GH levels are not decreased after tamoxifen or E2 treatment of ovariectomized-hypothyroid rats (see above). However, much of the somatotropic effects of GH are due to induction of IGF-I in the liver and other tissues, and recent reports have indicated that tamoxifen and E2 can suppress serum IGF-I levels (Huynh et al., 1993; Borski et al., 1996). Indeed, E2 was reported to decrease IGF-I even while increasing serum GH levels (Borski et al., 1996). Therefore, serum IGF-I levels were measured to determine whether tamoxifen attenuated T3 or GH actions to induce IGF-I. As shown in figure 4, both GH and T3 increased serum IGF-I levels in hypothyroid rats: however, T3-evoked a 3-fold increase in serum IGF-I that was highly significant whereas GH produced only a 55% increase that was not statistically significant. The weak response of IGF-I to GH compared to T3 is consistent with the lack of a somatotropic response to GH during the last 9 days of the experiment (see figs. 2 and 3). Tamoxifen alone had little effect on serum IGF-I levels, but enhanced GH actions to increase IGF-I: thus, in the presence of tamoxifen, GH elicited a significant 63% increase in serum IGF-I levels relative to vehicle controls. However, tamoxifen inhibited T3 effects to increase serum IGF-I levels
by 75%. The data indicate that tamoxifen inhibits T3 effects to increase serum IGF-I levels without decreasing basal IGF-I levels or altering GH actions to increase IGF-I in the absence of T3.

Left kidney dry mass was measured to provide an index of anabolic effects to increase lean body mass (fig. 5). Surprisingly, GH failed to affect kidney growth in hypothyroid rats in this study despite its effects to significantly increase body weight and tibia length. However, T3 produced a robust 69% increase in absolute kidney dry mass, and this response was inhibited by 47% by tamoxifen. Tamoxifen alone had no effect on kidney mass. T3 also increased relative kidney mass (mg dry weight per kg body weight) indicating that T3 effects to evoke increased renal growth exceeded its effects to promote overall somatic growth. Interestingly, tamoxifen did not inhibit T3 effects to increase relative kidney mass (fig. 5). This suggests that tamoxifen effects to inhibit absolute kidney mass were secondary to its actions to inhibit overall somatic growth.

**Tamoxifen interactions with T3 and GH effects on fat mass and serum triglycerides.** In rats, both E2 and tamoxifen have well recognized actions to decrease fat mass in association with their effects to decrease somatic growth. Thus, it was of interest to determine whether such tamoxifen effects would also be T3 dependent, and if decreases in fat mass would be proportional to decreases in somatic growth.

In addition, E2 and tamoxifen effects to increase triglycerides in the rat are T3 dependent (DiPippo et al., 1995; DiPippo and Powers, 1997), and we wished to examine tamoxifen and GH interactions on this parameter. As shown in figure 6, GH decreased absolute parametrial fat mass (g) in hypothyroid rats by 45% whereas T3 produced a 48% increase. When relative fat mass was determined (g parametrial fat mass per kg body weight) it was observed that GH effects were essentially unchanged whereas T3 no longer had an effect. Thus, T3 actions to increase fat mass were directly proportional to its actions to increase body weight. These results indicate major differences between GH and T3 in their effects on adipose tissue, and suggest that adipose tissue growth in the rat is T3 dependent.

Tamoxifen alone had no effect on either absolute parametrial fat mass or relative fat mass (fig. 6). However, tamoxifen significantly potentiated GH effects to decrease both absolute and relative fat mass. Indeed, the parametrial fat pads of GH plus tamoxifen-treated rats appeared to be devoid of stored lipids. Conversely, tamoxifen completely inhibited T3 effects to increase absolute fat mass, and further produced significant decreases in both absolute and relative fat mass compared to rats treated with vehicle or tamoxifen alone. Thus, tamoxifen effects to decrease adipose tissue mass were completely dependent on interactions with either GH or T3, and tamoxifen appeared to inhibit T3 effects although potentiat-
The data suggest that T3 produces effects to both promote and inhibit fat storage by multiple actions that may be both GH-independent and dependent, and that tamoxifen may dramatically tip the balance toward depletion of fat mass.

Neither T3 nor GH alone significantly affected serum triglyceride levels (fig. 6), although T3 displayed a tendency to decrease levels. Tamoxifen alone also had no effect on serum triglycerides, and also did not affect levels when coadministered with GH. However, as previously reported, coadministration of tamoxifen with T3 led to a 2.5-fold increase in serum triglycerides relative to rats receiving T3 alone. Thus, the effect of tamoxifen plus T3 to decrease parametrial fat mass was associated with increases in serum triglycerides, whereas the effect of GH to decrease parametrial fat mass did not affect serum triglyceride levels in either the presence or absence of tamoxifen.

**Effect of tamoxifen, GH and T3 on food intake, serum glucose levels and body temperature.** E2 and tamoxifen are well recognized to produce transient alterations in food intake (Tartelin and Gorski, 1973; Wade and Gray, 1979; Wade and Heller, 1993), and it is possible that alterations in somatic growth and fat mass may be secondary to alterations in caloric intake. To address this possibility, food intake was measured on days 7, 14 and 28. As shown in figure 7, T3 alone increased absolute food intake (g) and relative food intake (g per kg body weight) compared to vehicle-treated rats throughout the experiment. When averaged (see inset to fig. 7) T3-treated rats had absolute food intakes that were 64% greater than vehicle controls, and relative food intakes that were 42% greater. GH alone tended to decrease food intake: when averaged, the absolute and relative food intakes of GH-treated rats were decreased by 11 and 21%, respectively, compared to controls. Tamoxifen produced a 9% decrease in absolute food intake, and a 5% decrease in relative food intake. Surprisingly, tamoxifen had no effect on T3 actions to increase either absolute or relative food intake (fig. 7). Indeed, relative food intakes were 14% higher in rats receiving tamoxifen plus T3 compared to rats receiving T3 alone. Tamoxifen also tended to prevent GH actions to de-
crease food intake. Overall, tamoxifen effects on somatic growth and fat mass were not associated with corresponding alterations in food intake.

Serum glucose levels were also measured to determine whether glucose availability or utilization might be altered by tamoxifen. Both GH and T3 exhibited a modest effect to increase serum glucose levels by about 20 to 25% that was not statistically significant (fig. 8). Tamoxifen alone also produced increases in blood glucose (+15%) that were not statistically significant, and this effect of tamoxifen appeared additive with the effects of GH and T3. Overall, blood glucose levels did not appear to display major alterations in any of the treatment groups.

Rectal body temperature of the rats was measured on days 10 and 22 of the experiment. As shown in table 1, both T3 and GH produced significant increases in body temperature on day 10, but on day 22 the increase in body temperature produced by GH was no longer significant whereas T3 continued to be effective. The increases in body temperature produced by T3 were significantly greater than those produced by GH on both days 10 and 22. Tamoxifen alone had no significant effect on body temperature, and did not inhibit GH or T3 effects. Indeed, on day 22, body temperatures of tamoxifen plus GH-treated rats were significantly elevated relative to vehicle controls whereas GH alone failed to produce significant increases.

Tamoxifen, GH and T3 interactions on tibia BMD. In the absence of tamoxifen, T3 produced a 5.5% decrease in proximal tibia BMD (fig. 9) relative to controls whereas GH produced a 4.0% increase. Neither of these changes reached statistical significance; however, the GH-treated group had significantly higher BMD than the T3-treated group (P < .05). It was also notable that T3-treated rats exhibited a strong trend toward decreased proximal tibia BMD despite the fact that their average calcium intakes (vis-à-vis food intakes) were at least 42% greater than control or GH-treated rats per kg body weight (see fig. 7). Tamoxifen had no significant effect on proximal tibia BMD in vehicle or GH-treated rats (4.1 and 6.0% increases, respectively) but produced a significant 13.1% increase in T3-treated rats. These data indicate that the magnitude of tamoxifen actions to increase proximal tibia BMD in the ovariectomized rat is T3 dependent. Rats treated with tamoxifen plus GH had significant increases in proximal tibia BMD relative to vehicle controls (P < .05) in an apparent additive interaction.

In contrast to the proximal tibia, diaphysis BMD did not notably respond to T3, tamoxifen or GH (right panel). This is consistent with a low proportion of cancellous bone in the diaphysis (<5%) compared to the proximal tibia (>20%) and the selective effect of estrogens on cancellous bone in the rat (Shen et al., 1993; Turner et al., 1994).

**Discussion**

Recent studies have shown that a subset of E2 and tamoxifen responses depend on T3 for expression, suggesting that such responses may arise by modulation of T3 actions (see Introduction). However, T3 effects on growth and metabolism in the rat reflect complex actions to directly alter the expression of multiple metabolic genes and induce GH, a powerful somatotropic and metabolic hormone. Thus, many T3 actions may be secondary to GH induction. Because E2 and tamoxifen do not lower serum GH in T3-treated rats (see Introduction), some of their T3-dependent effects may reflect modulation of GH actions rather than T3 actions. The present study evaluated this question by comparing tamoxifen effects on responses evoked by T3 vs. GH in hypothyroid rats (deficient in both T3 and GH). Tamoxifen was used since it fully mimics E2 in T3-dependent responses, and in the absence of T3 produces less induction of GH than E2 (DiPippo and Powers, 1991; DiPippo et al., 1995).

In our study GH was poorly effective in eliciting somatic growth compared to T3. Nonetheless, tamoxifen did not inhibit GH-evoked weight gain whereas T3 effects were markedly inhibited (see fig. 3). Similarly, GH-evoked increases in tibia length and serum IGF-I were less than evoked by T3, yet tamoxifen was ineffective in inhibiting these GH effects whereas T3-evoked increases were strongly inhibited. Finally, GH and T3 produced opposite effects on fat mass and BMD. GH decreased absolute fat mass (g) and tended to increase BMD, and these GH effects were potentiated rather than inhibited by tamoxifen. In contrast, T3 increased absolute fat mass and tended to decrease BMD, and these effects were inhibited rather than potentiated by tamoxifen. Thus, the well-known ability of tamoxifen to decrease fat mass and increase BMD in ovariectomized rats exhibits characteristics of a T3-dependent phenomenon, and this is likely to also be the case with E2. Tamoxifen effects on fat and BMD are of

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**TABLE 1**

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Body Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 10</td>
<td>Day 22</td>
</tr>
<tr>
<td>Control</td>
<td>35.5 ± 0.3</td>
</tr>
<tr>
<td>GH</td>
<td>36.2 ± 0.1a</td>
</tr>
<tr>
<td>T3</td>
<td>36.9 ± 0.1b</td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>35.6 ± 0.1</td>
</tr>
<tr>
<td>Tamoxifen + GH</td>
<td>36.2 ± 0.2a</td>
</tr>
<tr>
<td>Tamoxifen + T3</td>
<td>36.9 ± 0.1b</td>
</tr>
</tbody>
</table>

Values show mean ± S.E. of groups of five to six rats. A group of six normal female rats (ovary-intact and no methimazole) had mean body temperatures of 37.0 ± 0.1 and 36.8 ± 0.1 °C on days 10 and 22, respectively.

a P < .05 vs. control.
b P < .05 vs. control or GH.

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**Fig. 8.** Effect of tamoxifen, GH and T3 on serum glucose levels in the ovariectomized-hypothyroid rat. Values represent the mean ± S.E. of five to six rats. None of the treatments significantly affected serum glucose levels.
particular interest because inhibition of GH actions, alone, would not be expected to produce the observed changes. Overall, tamoxifen was not observed to inhibit any of the GH responses observed whereas multiple T3 responses were inhibited. The data are consistent with tamoxifen and E2 producing multiple effects to decrease somatic growth and alter metabolism via modulation of T3 actions as opposed to GH actions. Such a mechanism is consistent with tamoxifen effects to inhibit T3 induction of pituitary GH and hepatic malic enzyme in the rat pituitary and liver (DiPippo and Powers, 1991, 1997; DiPippo et al., 1995). These T3 responses are mediated directly at the target gene (Glass et al., 1987; Petty et al., 1990; Samuels et al., 1988), and are unlikely to involve secondary mediators, such as GH, that could be modulated by tamoxifen or E2. E2 and tamoxifen also appear to mimic one another in inhibiting T3-induction of a novel glucocorticoid-binding protein (LAGS) in the rat liver (López-Guerra et al., 1997).

The poor efficacy of GH alone in producing continuous increases in body weight in our study is consistent with other hypothyroid rat models in which T4 produced greater somatic growth than exogenous GH (Scow et al., 1949; Nanto-Salonen et al., 1993; Lewinson et al., 1994). However, T3 increased fat mass, whereas GH decreased fat mass. Thus, body weight changes might be expected to underestimate increases in lean mass and longitudinal growth in GH-treated animals relative to T3-treated rats. Nonetheless, GH-evoked increases in tibia length were only 30% that of T3, and GH failed to increase kidney weight (an index of lean body mass) whereas T3 increased kidney weight by 70%. The results are consistent with studies showing that T4 can induce some longitudinal growth in the absence of GH, and that T4 is required for GH to fully manifest its somatotropic potential (Simpson et al., 1950; Asling et al., 1954; DeGroot, 1963; Thorngren and Hansson, 1973). The data support the notion that tamoxifen may alter rat growth, in part, by inhibiting T3 actions to evoke direct somatotropic effects or potentiate GH. This model has clinical parallels since T3 also is essential for normal somatotropic responses to GH in children (see Fisher and Polk, 1995).

Tamoxifen inhibited T3-evoked increases in serum IGF-I without altering modest GH effects on IGF-I. Tamoxifen effects on serum IGF-I may explain why tamoxifen can selectively suppress T3-evoked growth without altering serum GH or affecting somatotropic responses elicited by exogenous GH. This finding is consistent with reports that E2 and tamoxifen decrease serum IGF-I in thyroid-intact rats (Huynh et al., 1993; Borski et al., 1996), as well as in humans (Clemmons et al., 1980; Pollak et al., 1992; Goodman-Gruen and Barrett-Conner, 1996). Tamoxifen effects to decrease IGF-I may reflect inhibition of T3 actions to potentiate GH induction of hepatic IGF-I production (Wolf et al., 1989).

Although vehicle or tamoxifen-treated rats lost GH responsiveness concurrently (day 21), weights of rats given GH alone subsequently declined whereas weights of GH plus tamoxifen-treated rats were stable. GH also lost its effect to increase body temperature during the latter phase of treatment, and tamoxifen preserved this response. Surprisingly, these “protective” actions of tamoxifen were associated with potentiation of GH-evoked decreases in fat mass. Thus, decreases in fat stores seem unlikely to account for weight loss during the latter part of GH treatment, and catabolism of other organ compartments (muscle) is likely. Normal or elevated GH levels are present in a number of hypercatabolic states with muscle wasting (surgery, sepsis, AIDS, etc.) (Bentham et al., 1993; Rodgers, 1996). The possibility that tamoxifen might exert protective effects in such states merits attention.

Although tamoxifen significantly inhibited T3 effects to increase absolute kidney weight (mg dry weight); tamoxifen did not alter T3 effects to increase relative kidney weights (mg/kg body weight). This may reflect two distinct T3 actions that are differentially sensitive to tamoxifen. One T3 effect may stimulate renal growth in proportion to increases in body weight, and may be related to other somatotropic effects of T3 that are inhibited by tamoxifen. A second T3 effect appears to involve a distinct action to increase relative kidney mass independent of changes in somatic growth: this effect appears to be resistant to inhibition by tamoxifen. Others have reported similar results. Marshall et al. (1993) found that T4 increased kidney mass in hypophysectomized rats without altering body weight or IGF-I. GH elevated serum and kidney IGF-I, and produced increases in kidney mass additive with T4 and proportional to body weight gain. Marshall et al. (1993) concluded that T4, unlike GH, produced increases in renal growth that were unrelated to IGF-I induction or overall somatic growth.

Tamoxifen had no effect on T3 actions to increase body weight changes; thus, the mechanism by which tamoxifen increases renal weight is distinct from that of GH. In rats treated with tamoxifen, increases in kidney weight were unrelated to serum IGF-I levels or somatic growth. As expected, GH also increased kidney weights above T4 alone, but not above T3 alone, which is consistent with other reports (Khan et al., 1990; Samuels et al., 1993; DiPippo et al., 1995). These T3 responses are mediated directly at the target gene (Glass et al., 1987; Petty et al., 1990; Samuels et al., 1988), and are unlikely to involve secondary mediators, such as GH, that could be modulated by tamoxifen or E2. E2 and tamoxifen also appear to mimic one another in inhibiting T3-induction of a novel glucocorticoid-binding protein (LAGS) in the rat liver (López-Guerra et al., 1997).

The poor efficacy of GH alone in producing continuous increases in body weight in our study is consistent with other hypothyroid rat models in which T4 produced greater somatotropic responses than exogenous GH (Scow et al., 1949; Nanto-Salonen et al., 1993; Lewinson et al., 1994). However, T3 increased fat mass, whereas GH decreased fat mass. Thus, body weight changes might be expected to underestimate increases in lean mass and longitudinal growth in GH-treated animals relative to T3-treated rats. Nonetheless, GH-evoked increases in tibia length were only 30% that of T3, and GH failed to increase kidney weight (an index of lean body mass) whereas T3 increased kidney weight by 70%. The results are consistent with studies showing that T4 can induce some longitudinal growth in the absence of GH, and that T4 is required for GH to fully manifest its somatotropic potential (Simpson et al., 1950; Asling et al., 1954; DeGroot, 1963; Thorngren and Hansson, 1973). The data support the notion that tamoxifen may alter rat growth, in part, by inhibiting T3 actions to evoke direct somatotropic effects or potentiate GH. This model has clinical parallels since T3 also is essential for normal somatotropic responses to GH in children (see Fisher and Polk, 1995).

Tamoxifen inhibited T3-evoked increases in serum IGF-I without altering modest GH effects on IGF-I. Tamoxifen effects on serum IGF-I may explain why tamoxifen can selectively suppress T3-evoked growth without altering serum
temperature or food intake. Previous T3 dose-response studies found that T3 effects on pituitary prolactin and serum TSH were also insensitive to E2 or tamoxifen (DiPippo et al., 1995). Such data highlight the selective nature of E2 and tamoxifen actions to modulate T3 effects. Tamoxifen effects on T3 metabolism, elimination and transport have not been examined in the rat. Nonetheless, changes in such pharmacokinetic parameters seem unlikely to explain tamoxifen effects on T3 actions because such mechanisms would be expected to alter tissue T3 levels and similarly influence all T3 effects rather than target selective responses. Moreover, in humans, tamoxifen yields only modest elevations in T4-binding globulin (+29%) and serum T4 (+14%), and does not change free T4 levels or serum TSH (Mamby et al., 1995).

Tamoxifen’s failure to decrease food intake in T3-treated rats is also noteworthy because it indicates that tamoxifen effects on growth and fat mass reflect altered metabolism rather than impaired nutrition. Indeed, relative food intake (g/kg body weight) in the presence of tamoxifen plus T3 was slightly increased compared to rats treated with T3 alone. This may also occur in ovariectomized, thyroid-intact rats. These data are consistent with a substantial body of work indicating that transient E2 and antiestrogen effects on food intake seem unlikely to explain chronic actions to retard growth and decrease fat mass (see Tartelin and Gorski, 1973; Wade and Gray, 1979; Wade and Heller, 1993).

T3-dependent effects of tamoxifen to decrease somatic growth and fat mass were associated with increased serum triglycerides, in agreement with earlier observations (DiPippo et al., 1995; DiPippo and Powers, 1997). Tamoxifen effects on triglycerides may be independent of changes in fat mass because tamoxifen decreased fat mass in GH-treated rats without increasing triglycerides. The T3 requirement for E2 and tamoxifen effects on triglycerides is paradoxical because T3 alone tends to decrease triglycerides (DiPippo et al., 1995; DiPippo and Powers, 1997; Ingbar, 1985). However, T3 has complex effects on lipid metabolism to increase cholesterol, fatty acid and triglyceride synthesis (Ingbar, 1985; Wilcox and Heimberg, 1991; Lee and Lardy, 1965; Mariash et al., 1980; Blennemann et al., 1995; Fukuda et al., 1992; Saffari et al., 1992), promote adipocyte differentiation and growth (see Ailhaud et al., 1992) and enhance fat mobilization and fatty acid β-oxidation (Debons and Schwartz, 1961; Stakkestad and Bremer, 1983; Oppenheimer et al., 1991; Jokinen et al., 1994; Mynatt et al., 1994). Modulation of selective T3 effects by tamoxifen may shift the balance between opposing T3 actions to yield the observed changes.

Thyrotoxicosis is a risk factor for osteoporosis, and rat and human studies suggest that T3 at or close to physiological levels may promote bone loss in estrogen-deficiency states (Mosekilde et al., 1990; Allain and McGregor, 1993; Schneider et al., 1994; Allain et al., 1995; DiPippo et al., 1995). In this study, physiological T3 replacement in hypothyroid rats did not significantly decrease proximal tibia BMD although a downward trend was evident. Nonetheless, tamoxifen significantly increased proximal tibia BMD only in T3-treated rats, suggesting that tamoxifen actions may partly reflect modulation of T3 effects. This is consistent with our previous report that E2 lacked effect on BMD in the absence of T3, but blocked T3-evoked decreases (DiPippo et al., 1995). It should be emphasized that T3-treatment increased food intake, and corresponding increases in calcium intake may have attenuated T3 actions to decrease BMD relative to controls. Previous work reporting BMD decreases with low T3 doses used thyroidectomized rats given water with 1% CaCl2 to maintain calcium balance (DiPippo et al., 1995).

GH tended to increase proximal tibia BMD in hypothyroid rats. This was the opposite of T3 actions, and indicates that this T3 response is unlikely to arise solely by GH induction. Tamoxifen effects to increase BMD in GH-treated rats may relate to previously discussed protective actions with respect to body weight and catabolic metabolism during the final week of GH treatment. However, tamoxifen effects to increase BMD in T3-treated rats may involve additional effects to inhibit T3 actions unrelated to GH. Another implication of the bone data is that GH effects on BMD may be T3 dependent. GH alone tended to increase BMD (despite low calcium intakes), whereas T3 treatment (which elevates GH secretion) tended to lower BMD. Further study of GH and T3 interplay on BMD may be warranted since GH effects on BMD are of potential therapeutic interest.

A second estrogen receptor (ERβ) has recently been identified (Kuiper et al., 1996). This raises the question of whether a differing involvement of ERα vs. ERβ might underlie T3-dependent and T3-independent estrogen responses, and the varying ability of tamoxifen to act as a full estrogen agonist or antiestrogen in such responses. Studies using target genes containing classic EREs indicate that E2 similarly activates transcription driven by either ERα or ERβ, and tamoxifen similarly inhibits E2 effects driven by either ERα or ERβ (Kuiper et al., 1996; Mosselman et al., 1996; Paech et al., 1997). Thus, such ERE targeted systems provide little evidence to support the notion that differing involvement of ERs or ERβ may contribute to the genesis of T3-dependent or independent estrogen responses. However, E2 can also regulate genes containing transcription factor AP-1 response elements (AP-1 elements) by evoking ER-binding to jun (an AP-1 subunit) rather than ERαs, and tamoxifen can fully mimic E2 actions at many of these target genes (see Webb et al., 1995). A recent report by Paech et al. (1997) indicates important differences between ERα and ERβ at target genes containing AP-1 elements. Tamoxifen and E2 similarly activated gene expression in transfection systems with a target gene containing an AP-1 element gene driven by ERα. In contrast, tamoxifen (as well as other antiestrogens), but not E2, activated gene expression in systems with AP-1 target genes driven by ERβ. It is unclear how the T3-dependent estrogen responses identified in the present physiological studies relate to current molecular models of ER action at AP-1 regulated target genes. Nonetheless, if AP-1 is a target of ER-TR interplay, then the data of Paech et al. (1997) suggest that only ERαs can support T3-dependent responses in which E2 and tamoxifen have equivalent abilities to evoke the response.

Acknowledgments

The authors gratefully acknowledge the skilled technical assistance of Eric Lofberg and Susan Goldstein in this work.

References


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