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Tamoxifen Stimulates Expression of the c-*fos* Proto-Oncogene in Rodent Uterus

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SUMMARY

Estrogens regulate the *in vivo* expression of the c-*fos* proto-oncogene in rat uterus, and this regulation appears to occur at the transcriptional level. This system thus provides the ability to study the *in vivo* effects of antiestrogens on specific gene expression in normal estrogen target tissue. Immature rats were treated with estradiol, tamoxifen, or other nonsteroidal antiestrogens, total uterine RNA was isolated, and c-*fos* transcript levels were monitored by blot analysis. Tamoxifen increases the 2.2-kilobase c-*fos* transcript approximately 20-fold in 6 hr. This effect is comparable in magnitude to that produced by estradiol, but the maximum response to the hormone occurs in 3 hr. c-*fos* induction is observed at doses of 0.1–10 mg/kg tamoxifen. The nonsteroidal antiestrogens nafoxidine, CI-628, and 4-hydroxytamoxifen also induce c-*fos* expression. The induction of c-*fos* by both estradiol and tamoxifen is blocked by the progestin medroxyprogesterone acetate. In addition to effects on c-*fos* mRNA, tamoxifen also increases uterine levels of c-*jun*, *jun*-B, and c-*myc* mRNAs. These results indicate that tamoxifen acts *in vivo* as an estrogen agonist for activating expression of cellular oncogenes in normal uterine tissue.

Pharmacological studies of nonsteroidal antiestrogens began in 1958 when Lerner *et al.* (1) described the properties of MER-25, the first agent in this class. Numerous laboratory and clinical studies of these agents were performed, and approximately 15 years ago use of the antiestrogen tamoxifen for treatment of breast cancer was begun in the United States (see Refs. 2 and 3 for recent reviews). Today, tamoxifen is a standard treatment for patients with primary or advanced breast cancer.

Tamoxifen is thought to produce its therapeutic effects primarily by competitive blockade of estrogen receptors in breast cancer cells, thereby producing an antiproliferative effect. In some situations, however, the drug exhibits estrogenic effects or mixed agonist/antagonist actions. The specific pharmacological activity of tamoxifen depends on a complex interplay between a number of factors, including differences in pharmacokinetics and metabolism in different species and under different treatment protocols, differences in the interactions of tamoxifen with estrogen receptors from different sources, and the specific pharmacological endpoint monitored (2–7).

Tamoxifen was originally selected for development and therapeutic use because of its efficacy and low incidence of side effects, relative to other nonsteroidal antiestrogens (2, 3, 8). Recently, however, the potential side effects associated with tamoxifen therapy for the treatment or prevention of breast cancer have received considerable attention (2, 3, 9). Several recent clinical studies have suggested a possible link between tamoxifen use and the development of endometrial carcinoma (10–14), presumably due to the estrogenic stimulation of the endometrium by the antiestrogen. An understanding of the actions of this drug on normal uterine tissue should aid in the analysis of this issue.

Many laboratories have investigated the actions of tamoxifen and related nonsteroidal antiestrogens such as nafoxidine and CI-628 in animal models such as the rat uterus. The majority of studies in this model have used endpoints such as uterine growth or histological changes and have often used multiple-dosage regimens. These studies have provided much useful information, but such endpoints are far removed from the initial drug-receptor interactions, and the interpretation of such studies may be complicated by changes in receptor levels and turnover (15, 16), the development of refractoriness when multiple hormone injections are used (17, 18), or other factors. We thus sought an endpoint to evaluate very early uterine responses to tamoxifen, to minimize the effects of such factors on our experimental results.

Recently we (19, 20) and others (21, 22) have shown that estrogens elevate the expression of several cellular oncogenes in normal rat uterus. One of these effects, the elevation of c-*fos* mRNA, seemed well suited for monitoring of early estrogenic actions of tamoxifen in rodent uterus. c-*fos* is the cellular analog

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ABBREVIATIONS: SDS, sodium dodecyl sulfate; SSC, standard saline citrate; kb, kilobase(s).

of the transforming oncogene originally isolated from a murine osteosarcoma-causing virus, and overexpression of either the viral or cellular oncogenes can cause transformation in experimental systems. The Fos protein is thought to act primarily by forming a heterodimer (AP-1) with proteins of the *jun* proto-oncogene family; AP-1 then functions as a transcriptional activator of genes containing AP-1 or tetradecanoylphorbol acetate-response elements (see Ref. 23 for a recent review).

This response was chosen as an experimental endpoint for this study because *fos* mRNA is rapidly and specifically elevated in the uterus after estradiol administration (19, 21) and because considerable experimental evidence suggests that this effect is due to direct transcriptional activation of the gene by the estrogen receptor (19–22). In addition, altered proto-oncogene expression is one potential mechanism that could contribute to abnormal uterine growth. Our results indicate that tamoxifen is a full agonist for the acute induction of c-*fos* expression in normal uterine tissue. We should point out, however, that there is not yet any direct evidence that c-*fos per se* plays a role in endometrial tumorigenicity. At this time, it may thus be more appropriate to consider *fos* as a "growth-related" or "immediate early" gene in the context of uterine biology.

Materials and Methods

Animals. Immature female Sprague-Dawley rats (21 days of age, 40–45 g; Harlan Sprague Dawley, Indianapolis, IN) were ovariectomized 4–7 days before use. Animals were injected subcutaneously in the periscapular region with 0.5 ml of 5% ethanol/95% saline containing estrogens or antiestrogens. Unless otherwise specified in the text or legends, doses of estradiol were 40 μg/kg of body weight and doses of tamoxifen were 1 mg/kg.

For the inhibitor studies, animals were treated with either a single dose of puromycin (100 mg/kg) 30 min before tamoxifen or two equal doses (4 mg/kg each) of actinomycin D 3 hr before and then simultaneously with the antiestrogen. This dose of puromycin effectively inhibits protein synthesis *in vivo* in both rat uterus (24) and liver (25). Both inhibitors were administered by intraperitoneal injection in 0.5 ml of vehicle.

For the studies with medroxyprogestrone acetate, animals were ovariectomized as described above and were allowed to recover for 1–2 days. To induce progesterone receptors, animals were then given priming doses of estradiol (40 μg/kg) on each of the next 2 days. The animals then remained untreated for an additional 2 days. On the next day animals were treated with estradiol or tamoxifen alone or with a combination of these agents plus medroxyprogesterone acetate, as described under "Results".

RNA preparation. Total uterine RNA was prepared by the method of Chirgwin *et al.* (26), as described previously (19, 27). Briefly, uteri were removed from anesthetized animals and immediately homogenized in 5 M guanidinium isothiocyanate, using a Polytron homogenizer (Brinkman, Westbury, NY) set at half-maximal power, for 60 sec. In most cases, uteri from two or three animals were pooled for the preparation of each RNA sample. RNA was pelleted through 5.7 M CsCl, extracted twice with phenol-chloroform (1:1) and once with chloroform, and precipitated with ethanol. RNA was quantified by absorbance at 260 nm.

Blot analysis. Samples of total RNA (10 μg) were denatured for 30 min in 15 mM methylmercuric hydroxide (Alfa, Salt Lake City, UT) and were separated on 1% agarose gels containing 6% (v/v) formaldehyde. The gels were stained with ethidium bromide after electrophoresis and the rRNA bands were visualized under UV light to ensure that constant amounts of RNA had been loaded in each lane. If differences in RNA levels were noted, the samples were not used for further analysis.

The RNA samples were then transferred to Duralon membranes (Stratagene, La Jolla, CA) by electroblotting in 25 mM sodium phosphate, pH 6.5. Membranes were allowed to dry at room temperature and were then prehybridized for 3 hr at 62° in 0.8 M NaCl, 2 mM EDTA, 0.5% SDS, 20 mM piperazine-*N*,*N*′-bis(2-ethanesulfonic acid), 50% deionized formamide, 100 μg/ml denatured salmon sperm DNA.

The ^{32}P-labeled c-*fos* antisense RNA probe was synthesized from the insert in a pGEM vector (Promega, Madison, WI) according to the manufacturer's directions, using Sp6 RNA polymerase, as described previously (19, 27), and the radiolabeled probe was added directly to the prehybridization mixture. This is a mouse c-*fos* probe originally obtained by digestion and subcloning of mouse *pc-fos*-3 (19). Blots were hybridized for 16–24 hr at 60°, washed twice at room temperature for 30 min with 2× SSC (1× SSC is 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0) containing 0.1% SDS, washed twice at 60° for 30 min each with 0.1× SSC containing 0.1% SDS, and then rinsed with 2× SSC. After the rinse, blots were treated for approximately 5 min with RNase A (1 μg/ml) in 2× SSC, washed for 10 min with 0.1× SSC containing 0.1% SDS, and then exposed to X-ray film.

Where indicated under "Results", films were scanned with a Zeineh Soft Laser scanning densitometer. Transcript levels in each figure or table are given in arbitrary absorbance units obtained from the scans. Values for each treatment group are expressed relative to the values of other samples run in the same experiment and analyzed on the same film. Therefore, values for any treatment group may be compared directly with values for all other samples in the same figure or table.

^{32}P-labeled riboprobes complementary to c-*jun*, *jun*-B, and c-*myc* were prepared and used to measure these transcripts in the uterine RNA samples as described previously (27).

Materials. [^{32}P]UTP (800 Ci/mmol) was obtained from Amersham Radiochemicals (Arlington Heights, IL) and was diluted to 400 Ci/mmol with radioinert UTP (Boehringer Mannheim) for the RNA polymerase reactions. Guanidine isothiocyanate, cesium chloride, and formamide were obtained from International Biotechnologies Inc. (New Haven, CT). Estradiol was obtained from Steraloids (Wilton, NH), medroxyprogesterone acetate was obtained from Sigma Chemical Co. (St. Louis, MO), and tamoxifen and CI-628 were kindly provided by Upjohn and Eli Lily, respectively. All other chemicals and reagents were purchased from Sigma and were of the highest grade commercially available.

Results

In our initial studies we treated animals with tamoxifen several times to determine whether the antiestrogen induces c-*fos* mRNA expression and, if it does, to define the time course of the effect. Fig. 1 shows a representative Northern blot of *fos* mRNA induction by tamoxifen at several time points, and Fig. 2A illustrates a composite time course obtained from several such experiments. It is clear from these results that tamoxifen increases levels of the 2.2-kb *fos* transcript and that a maximum response to the antiestrogen occurs approximately 6 hr after

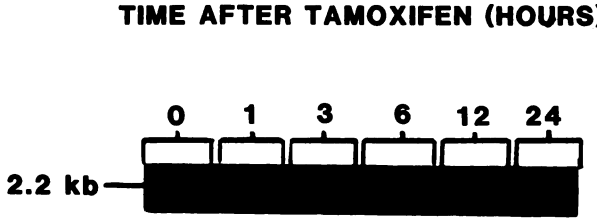

Fig. 1. Induction of c-*fos* mRNA by tamoxifen. Animals were treated with tamoxifen (1 mg/kg) for the indicated times before sacrifice. Total uterine RNA was prepared and analyzed by blot analysis as described in Materials and Methods. Samples in each *lane* were prepared from two or three pooled uteri. Two separate samples are shown for each time point. The position of the 2.2-kb c-*fos* transcript is indicated.

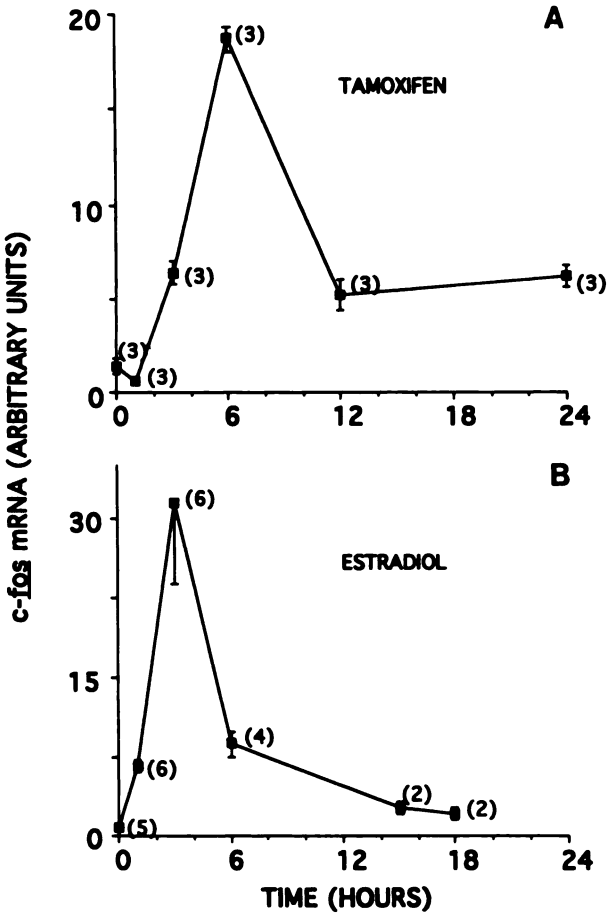

Fig. 2. Time course of c-*fos* mRNA induction by tamoxifen. A, Groups of animals were treated with tamoxifen (1 mg/kg) for the indicated times before sacrifice and analysis of c-*fos* mRNA levels as described in Materials and Methods. Values are in arbitrary units based upon densitometric scans of the resultant films, with the indicated standard errors; the numbers of determinations are given in parentheses. B, For comparison, a separate group of animals were treated with estradiol and analyzed for c-*fos* mRNA levels in a similar manner.

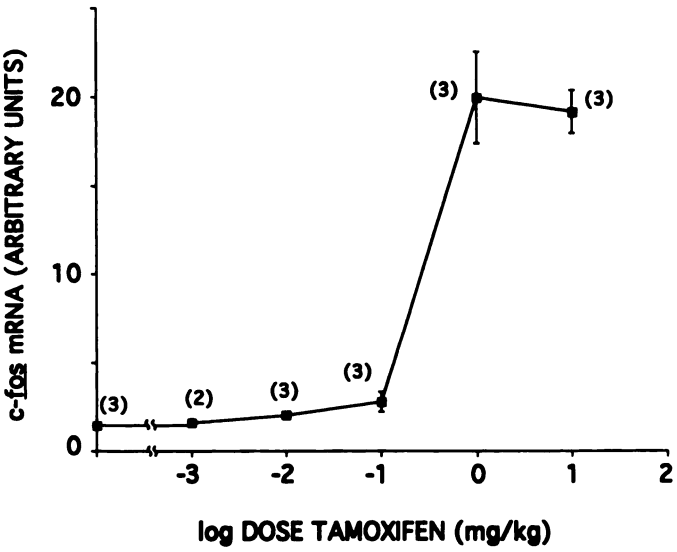

Fig. 3. Dose-response curve for induction of c-*fos* mRNA by tamoxifen. Groups of animals were treated for 6 hr with the indicated doses of tamoxifen, and c-*fos* mRNA levels were determined as described in Materials and Methods. Values are in arbitrary units based upon densitometric scans of the resultant films, with the indicated standard errors; the numbers of determinations are given in parentheses.

TABLE 1

Induction of c-*fos* mRNA by estradiol and tamoxifen

Animals were treated with estradiol for 3 hr or with tamoxifen for 6 hr before sacrifice; control animals received the vehicle alone. Total uterine RNA was analyzed for c-*fos* mRNA by blot analysis, as described in Materials and Methods. The levels of c-*fos* mRNA are based on densitometric scans of the resultant films and are expressed in arbitrary units, as described in Materials and Methods. Values represent means ± standard errors of *n* determinations; two or three uteri were pooled to prepare the samples used for each determination.

Treatment	*n*	c-fos mRNA level
		units
Control	26	4.59 ± 1.1
Estradiol (3-hr treatment)	20	100 ± 6.4
Tamoxifen (6-hr treatment)	25	101 ± 5.8

TABLE 2

Effect of actinomycin D on tamoxifen induction of c-*fos* mRNA levels

Groups of animals were treated for 6 hr with tamoxifen, actinomycin D, the two agents together, or the vehicle alone, as described in Materials and Methods. Total uterine RNA was analyzed for c-*fos* mRNA levels by blot analysis and densitometric scans. Values are in arbitrary units, as described in Materials and Methods, and represent means ± standard errors of *n* determinations; two or three uteri were pooled to prepare the samples for each determination.

Treatment	*n*	c-fos mRNA level
		units
Control	3	0.55 ± 0.06
Actinomycin D	3	3.7 ± 1.8
Tamoxifen	3	100 ± 16
Tamoxifen + actinomycin D	3	12.4 ± 4.3

treatment. This is slightly slower than the maximum response to estradiol, which occurs at 3 hr (Fig. 2B) (19, 21).

Given the time course observed in Fig. 2A, we determined the dose-response curve for the induction of uterine c-*fos* levels 6 hr after tamoxifen treatment, i.e., when the response to the antiestrogen is maximal. As seen in Fig. 3, a dose of 1 mg/kg of body weight produces a maximum response in levels of the proto-oncogene transcript.

To compare the efficacy of estradiol and tamoxifen, we measured the magnitude of *fos* induction in a large number of samples, either 3 hr after estrogen administration or 6 hr after tamoxifen administration, i.e., the respective times at which peak responses to the two agents occur. The results of these studies are shown in Table 1 for several dozen measurements, using two or three pooled uteri for each determination. It is clear that tamoxifen and estradiol are equally efficacious for the induction of c-*fos* mRNA levels.

Previous studies established that the increase in uterine c-*fos* mRNA seen after estradiol treatment is due at least in part to transcriptional activation (19–22). We therefore examined the effects of RNA and protein synthesis inhibitors on induction by tamoxifen of the proto-oncogene transcript. The results of these studies are shown in Tables 2 and 3 for actinomycin D and puromycin, respectively. Actinomycin D completely blocks induction by tamoxifen, and the inhibitor itself is without significant effect (Fig. 2). Puromycin alone may cause a very slight increase in c-*fos* mRNA levels, but it is clear that this protein synthesis inhibitor significantly reduces the in-

TABLE 3

Effect of puromycin on tamoxifen induction of c-*fos* mRNA levels

Groups of animals were treated for 6 hr with tamoxifen, puromycin, the two agents together, or the vehicle alone, as described in Materials and Methods. Total uterine RNA was analyzed for c-*fos* mRNA levels by blot analysis and densitometric scans of the resultant films. Values are in arbitrary units, as described in Materials and Methods, and represent means ± standard errors of *n* determinations; two or three uteri were pooled to prepare the samples for each determination.

Treatment	*n*	c-*fos* mRNA level
		units
Control	4	5.2 ± 1.7
Puromycin	4	10.4 ± 4.9
Tamoxifen	6	100 ± 10.1
Tamoxifen + puromycin	6	47.0 ± 4.6

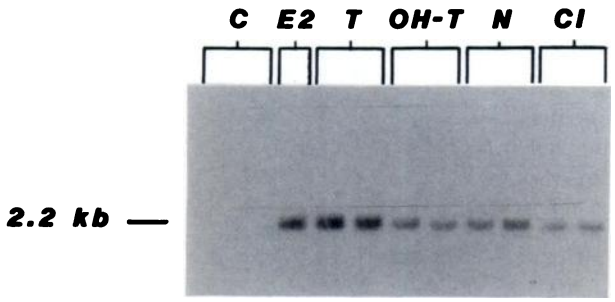

Fig. 4. Induction of c-*fos* mRNA by various nonsteroidal antiestrogens. Groups of animals were treated with 1 mg/kg tamoxifen (*T*), 4-hydroxytamoxifen (*OH-T*), nafoxidine (*N*), CI-628 (*CI*), or the vehicle alone (*C*) for 6 hr before sacrifice. Total uterine RNA was prepared and analyzed for the 2.2-kb c-*fos* transcript as described in Materials and Methods. For comparison, a sample prepared from animals treated in parallel with estradiol for 3 hr (*E2*) was run on the same gel. Each *lane* represents a separate sample prepared from three pooled uteri.

crease in transcript levels seen after antiestrogen treatment (Table 3).

At this point we sought to determine whether other nonsteroidal antiestrogens induce c-*fos* mRNA levels in a manner similar to that of tamoxifen. For this purpose, we treated groups of animals with tamoxifen, 4-hydroxytamoxifen, nafoxidine, and CI-628, prepared total uterine RNA 6 hr later, and examined *fos* transcript levels by blot analysis. The results of this experiment are shown in Fig. 4. All four compounds clearly increase levels of c-*fos* mRNA, indicating that this response is not unique for tamoxifen but is a general uterine response to nonsteroidal antiestrogens. Although the magnitude of the responses to the different antiestrogens varies in this experiment, we are not certain that the conditions used (e.g., times, doses, etc.) yield a maximum response for compounds other than tamoxifen.

The results presented above indicate that tamoxifen behaves as an estrogen agonist for the induction of c-*fos* mRNA levels in the rodent uterus. Because progesterone blocks the induction by estradiol of this proto-oncogene message (28), we investigated whether a progestational agent would also block the response caused by tamoxifen. For these studies, animals were treated with estradiol or tamoxifen alone or with these agents in combination with medroxyprogesterone acetate. The results of this experiment (Table 4) demonstrate that medroxyprogesterone acetate blocks the induction of *fos* mRNA by both the estrogen and the antiestrogen.

Because estradiol induces expression of many cellular oncogenes in the uterus (29), we also examined the effect of tamoxifen on uterine levels of several other proto-oncogene mRNAs,

TABLE 4

Inhibition of estrogen and antiestrogen induction of c-*fos* mRNA by medroxyprogesterone acetate

Estrogen-primed rats were treated with estradiol (E_2) or tamoxifen (TAM) alone or were pretreated with 2.5 mg of medroxyprogesterone acetate (MPA) for 1 hr before receiving either agent. Control animals were treated with the vehicle alone. Animals were sacrificed 3 hr after treatment with estradiol or 6 hr after treatment with tamoxifen. Total uterine RNA was analyzed for the 2.2-kb c-*fos* transcript by blot analysis, as described in Materials and Methods, and levels are expressed in arbitrary units based upon densitometric scans of the resultant films. Values are the means ± standard errors of the number of determinations in parentheses.

Sample	c-*fos* mRNA level	Inhibition
	units	%
Control	0	
E_2 alone	26.5 ± 1.5 (5)	
E_2 + MPA	7.6 ± 0.9[a] (4)	71
TAM alone	25.7 ± 5.0 (5)	
TAM + MPA	9.1 ± 2.2[b] (3)	65

[a] $p < 0.01$, versus estradiol alone.
[b] $p < 0.05$, versus tamoxifen alone.

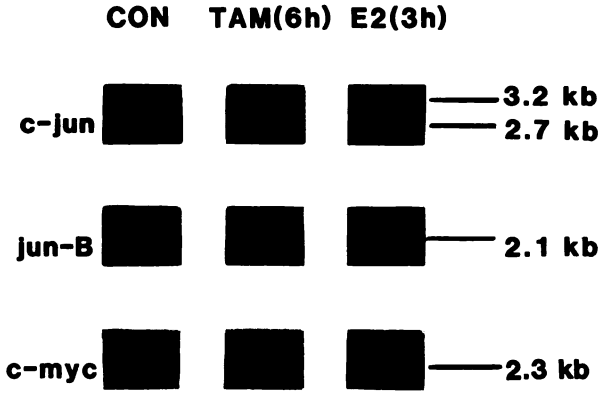

Fig. 5. Induction of c-*jun*, *jun*-B, and c-*myc* mRNAs by tamoxifen. Groups of animals were treated with tamoxifen (*TAM*) (for 6 hr), estradiol (*E2*) (for 3 hr), or the vehicle alone (*CON*). Total uterine RNA was prepared and analyzed for proto-oncogene transcripts using specific antisense RNA probes, as described in Materials and Methods. Each *lane* represents a separate sample prepared from three pooled uteri.

including c-*jun*, *jun*-B, and c-*myc*. The data in Fig. 5 indicate that the antiestrogen increases uterine levels of the 3.2- and 2.7-kb c-*jun* transcripts, the 2.1-kb *jun*-B transcript, and the 2.3-kb c-*myc* transcript, as well as the 2.2-kb c-*fos* message. The induction of *jun* and *myc* mRNAs by tamoxifen is not as great as that by estradiol, but we made no attempt to maximize these specific responses to the antiestrogen, and measurements under different experimental conditions might produce quantitatively different results.

Discussion

In this report we have used induction of the nuclear proto-oncogene c-*fos* as an endpoint to assess the estrogenic activity of tamoxifen in normal uterine tissue in a laboratory model of estrogen-induced growth. In the rat uterus c-*fos* mRNA levels are rapidly induced by estradiol, with a doubling of transcript levels within 30 min and a 20–40-fold increase within 3 hr (19, 21). This is one of the most rapid and dramatic increases in estrogen-stimulated gene expression observed to date in animal models of uterine growth. This response is thus an excellent marker to assess early tissue effects of estrogens and antiestrogens, because it minimizes potential complications due to turnover or resynthesis of hormone receptors or other local or

systemic effects that may influence tissue responses at longer times.

The results in this test system clearly indicate that tamoxifen is a full estrogen agonist, on the basis of acute induction of c-*fos* mRNA levels. This effect of tamoxifen is completely prevented by actinomycin D, indicating that *de novo* synthesis of the 2.2-kb *fos* transcript occurs. The partial blockade by puromycin suggests that this effect is due at least in part to transcriptional activation, rather than being a secondary effect resulting from the synthesis of an intermediate mRNA and protein product. The mechanism of *fos* induction by tamoxifen thus appears to be similar to that of induction by estradiol (19, 21). This is also consistent with the observation that medroxyprogesterone acetate blocks the induction by both estrogen and antiestrogen. Despite these observations, additional studies (e.g., nuclear run-on and transient promoter/reporter assays) will be required to establish unequivocally the role of transcriptional and post-transcriptional mechanisms in c-*fos* induction by tamoxifen.

The major difference in the uterine induction of c-*fos* mRNA after treatment with either estradiol or tamoxifen is the longer time required for a maximum response to the antiestrogen. A slightly delayed response to nonsteroidal antiestrogens has been observed in other studies (15, 16). This could be due to differences in the distribution of the two compounds or the biotransformation of antiestrogens to compounds (e.g., 4-hydroxytamoxifen) with greater affinity for the estrogen receptor (6, 15, 16).

In addition to c-*fos* induction, tamoxifen increases uterine levels of c-*jun*, *jun*-B, and c-*myc*. Others have shown that, in immature rats, a single dose of tamoxifen also increases complement component C3 (30) and ornithine decarboxylase activity (31) within 24 hr. Based upon these acute changes in individual mRNAs and proteins, the early effects of tamoxifen on the rat uterus appear to be fully estrogenic, at least in a qualitative sense. This is consistent with previous observations that tamoxifen alone can elicit estrogenic responses in the rat uterus but that the drug can antagonize the uterine response to estradiol when the two agents are given simultaneously (3, 5, 6). This suggests that the pharmacological classification of tamoxifen (i.e., estrogen agonist, estrogen antagonist, or partial estrogen agonist) depends upon the treatment regimen used and the specific endpoint measured. It is thus difficult to categorize the drug as either a pure estrogen or antiestrogen in the rat uterus.

In other systems it has been shown that estrogenic and antiestrogenic properties of tamoxifen are gene specific (32), that antiestrogen-receptor complexes do not activate transcription in a manner similar to that of estrogen-receptor complexes (33), and that antiestrogen-receptor and estrogen-receptor complexes exhibit different physical properties (34). Thus, it would be premature to speculate that tamoxifen stimulates all estrogen-responsive genes in the uterus, especially because the overall effects of nonsteroidal antiestrogens on uterine morphology and cell growth are quite distinct from those produced by estradiol (15, 16). In fact, one possible explanation for the atypical uterine growth response to tamoxifen is that the drug stimulates expression of some uterine genes (e.g., c-*fos*) but does not activate (or represses) others. The net result could be an "imbalance" in the levels of c-Fos, additional members of the Fos/Jun families, or other transcription factors and growth regulators. Such an altered profile of key intracellular molecules could contribute to the development of abnormal growth and differentiation properties of uterine cells. Although purely hypothetical, an imbalance of growth modulators is one potential mechanism for the increased incidence of endometrial carcinoma seen in patients receiving long term tamoxifen therapy (9–14).

Other groups have studied the estrogenic activity of tamoxifen in human endometrial carcinoma cells. The antiestrogen can stimulate the growth of endometrial carcinoma cells in culture (35, 36) and of tumors carried in nude mice (37–40). One such study documented that tamoxifen and estradiol both increased c-*fos* mRNA levels in human endometrial tumors grown in nude mice (40), although the response to the antiestrogen was roughly half that produced by the hormone. Other recent studies also found that tamoxifen stimulated the growth of human endometrial cancer cells in culture, but the studies reported different effects of 4-hydroxytamoxifen and estradiol on transforming growth factor gene expression (36). These differences were dependent upon the specific cell lines and culture conditions used (36). Our results and the numerous reports of others thus indicate that tamoxifen has agonist activity in both normal uterine tissue and uterine tumor cells.

Studies in nude mice have emphasized that tamoxifen can have opposing actions in different tissues of the same animal. For example, when both breast cancer and uterine carcinoma cells are transplanted into the same animal, tamoxifen inhibits the growth of the breast cancer cells but stimulates the growth of the endometrial cancer cells (39). This indicates that the activity pattern of tamoxifen is dependent upon cellular factors, and this emphasizes the importance of evaluating estrogenic and antiestrogenic endpoints in the specific cell types of interest.

In reference to the development of endometrial carcinoma in women receiving tamoxifen, we found that medroxyprogesterone acetate blocks the acute induction of c-*fos* mRNA by both tamoxifen and estradiol. If the rat model has relevance to the development of the human disease, this may suggest that progestational agents should be included in tamoxifen treatment regimens much as they are now included in long term estrogen replacement therapy for postmenopausal women. It is interesting to note that most studies demonstrating an increased incidence of endometrial carcinoma in women receiving tamoxifen monitored patients who were not simultaneously receiving a progestational agent (9–14).

Tamoxifen is a main-line agent for the pharmacological management of breast cancer, and many workers have studied the growth-inhibitory effects of this agent on breast cancer cells (2, 3). Much less is known, however, about the effects of this antiestrogen on the expression of genes likely to be involved in the growth of normal uterine cells. This information is important for evaluation of the role that tamoxifen plays in the etiology of endometrial carcinoma. A thorough understanding of the effects of tamoxifen on the uterus will require an understanding of the intrinsic activity of the compound for activation of specific genes, tissue-specific factors, effects on estrogen receptor levels and turnover, biotransformation, and other pharmacological parameters. The rat model of rapid proto-oncogene expression that we have studied in this work seems particularly well suited for determination of the intrinsic activity of tamoxifen for activation of estrogen-responsive genes in

the uterus and may thus contribute to our overall understanding of antiestrogen actions in normal tissue.

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