Direct Effects of Tamoxifen on Growth Hormone Secretion by Pituitary Cells *in vitro*

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There is now strong evidence to suggest that insulin-like growth factor I (IGF-I) plays an important role in breast cancer proliferation. Recently we observed that tamoxifen-treated stage I breast cancer patients have serum IGF-I levels significantly lower than placebo-treated patients. Since IGF-I is growth hormone (GH) dependent, we have tested the hypothesis that tamoxifen alters serum IGF-I levels through direct inhibition of GH secretion. Immature lamb pituitary cultures were examined for acute (3 h) or chronic (1–6 day) effects of the drug, using doses (0.1–10 μmol/l) based on known steady state levels in patients on tamoxifen therapy (0.31–3.1 μmol/l). Tamoxifen had a direct, dose-related, inhibitory effect on GH release from pituitary somatotropes, during acute as well as chronic treatment. The 10 μmol/l dose consistently decreased both basal and growth hormone releasing factor stimulated GH release. These *in vitro* data are consistent with our hypothesis that tamoxifen suppresses serum IGF-I levels by acting at the pituitary to inhibit GH release.

INTRODUCTION

THERE IS now strong evidence to suggest that insulin-like growth factor I (IGF-I) plays an important role in breast cancer [1–5]. Recently, we observed that circulating IGF-I levels were significantly lower in patients with stage I breast cancer on tamoxifen treatment as compared with placebo-treated patients (0.9 vs. 1.4 U/ml; $P < 0.002$) [6]. Our study confirmed the preliminary findings from a smaller, non-randomised study (0.48 vs. 1.03 U/ml; $P < 0.01$) and supported the suggestion that the anti-neoplastic effects of tamoxifen might be, at least in part, due to suppression of circulating IGF-levels [7].

The mechanism by which tamoxifen, a synthetic nonsteroidal antioestrogen [8], exerts this inhibitory effect is not clear. However, the bulk of the evidence, from clinical as well as experimental studies, indicates that the major influence of oestrogens on serum IGF-I levels is indirect, by modulating growth hormone (GH) secretion and biological activity [9–13]. Therefore, we decided to test the hypothesis that tamoxifen is suppressing serum IGF-I levels by acting at the level of the pituitary to inhibit GH release.

Our laboratory has been using cultures of immature lamb pituitary cells to investigate the mechanisms that regulate GH secretion [14, 15]. We have chosen this test system to examine both acute and chronic effects of tamoxifen, on basal as well as growth hormone releasing factor (GRF) stimulated and somatostatin inhibited GH release. To determine the specificity of tamoxifen actions, prolactin (PRL) and luteinising hormone (LH) were also assayed. The doses tested (0.1–10 μmol/l) were based on known steady state drug levels in breast cancer patients receiving 20–40 mg of tamoxifen per day (0.31–3.1 μmol/l) [16].

MATERIALS AND METHODS

Materials

Collagenase (Type IV) was purchased from Cooper Biomedical (Mississauga, Ont., Canada), DNase from the Sigma Chemical Co. (St Louis, Missouri), 24-well (2 cm²) tissue culture plates and Ham's F-10 culture medium from Flow Laboratories, Inc. (Mississauga, Ontario, Canada), defined fetal bovine serum (FBS) from Hyclone Sterile Systems (Logan, Utah), penicillin-

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G from Ayerst (Montreal, Quebec, Canada), gentamycin from Schering (Pointe-Claire, Quebec, Canada), and fungizone from Squibb (Montreal, Quebec, Canada). Growth hormone releasing factor (hGRF[1-44]) and somatostatin[1-14] were obtained from Peninsula Laboratories (Belmont, California). Tamoxifen citrate (trans isomer only) was a gift from ICI Pharmaceuticals (Macclesfield, Cheshire, UK).

Cell cultures

Anterior pituitary glands were collected from 21–33 day old immature male lambs (mixed breed: Suffolk/Dorset/Hampshire) following anesthesia with 50 mg/kg of ketamine and exsanguination. These protocols were in accordance with CCAC guidelines and had approval from the McGill University Animal Care Committee. Individual glands were minced with sterile scissors and dispersed enzymatically using collagenase and DNase [14, 15]. Monolayer cultures (100,000 cells/well.ml) were incubated at 37°C in a humidified atmosphere containing 5% CO_2 for 72 h before experiments were begun. The culture medium (Ham's F-10 supplemented with 10% heat-inactivated FBS and antibiotics (200 U/ml penicillin, 5 µg/ml fungizone and 32 µg/ml gentamycin) was changed daily beginning on the second day of culture. This medium contained ≤ 1.5 pg of oestrogens (oestradiol + oestrone) and ≤ 3.5 pg of androgens (testosterone + androstenedione) per ml. The phenol red concentration was 3.1 nmol/ml.

Experimental protocols

The ovine pituitary cells were examined for acute effects of tamoxifen on basal as well as GRF-stimulated and somatostatin-inhibited GH secretion, basal PRL and basal LH release. 3, 6 or 9 days after the cultures were established, the cells were preincubated for 1 h in culture medium, washed and then incubated for 3 h at 37°C in culture medium supplemented with varying concentrations of tamoxifen (0.1–10 µmol/l) or the equivalent volume of diluent (methanol) and 1 nmol/l GRF, 100 nmol/l somatostatin or a combination of the two hypothalamic regulatory peptides. Previous studies using this test model had shown that these doses of GRF and somatostatin had maximal stimulatory and inhibitory effects, respectively, on GH release [14, 15].

Cultures were also tested for chronic effects of tamoxifen using a 3 or 6 day treatment protocol (on days 3–6 or 9 in vitro). Media were collected and changed every 24 h, and test solutions (culture medium supplemented with 0.1–10 µmol/l tamoxifen or equivalent concentrations of the diluent) were made up fresh daily. At the end of the 3 or 6 days of pretreatment, the cultures were rinsed and preincubated in culture medium alone for 1 h, then rinsed and incubated for 3 h at 37°C in culture medium to which 1 nmol/l GRF, 100 nmol/l somatostatin or a combination of the two peptides had been added.

Each experimental group was tested in triplicate or quadruplicate; all of the acute and chronic series of experiments were carried out using 3–6 different preparations of pituitary cells, with final results calculated from the means of the repeat experiments. Media samples were collected, centrifuged for 10 min at 3000 rpm at 4°C and the supernatants were kept frozen at −20°C until assayed for ovine GH and, in certain cases, for ovine PRL and LH as well. Cell cultures were rinsed with cold phosphate-buffered saline and then frozen at −20°C until representative wells were processed for DNA and protein determinations.

Table 1. Effect of tamoxifen during 3 h basal LH, GH and PRL secretion

	LH	GH	PRL
Control (with or without diluent)	0.31 (0.03)*	23.4 (1.5)	31.8 (2.7)
Tamoxifen (0.1 µmol/l)	0.31 (0.03)	24.7 (3.0)	28.0 (2.1)
(1 µmol/l)	0.28 (0.01)	22.9 (3.5)	21.1 (2.5)†
(10 µmol/l)	0.29 (0.02)	13.1 (1.7)†	8.4 (1.1)†

* Data are expressed as ng/ml, mean (S.D.), $n = 4$ wells/experimental group.
† $P < 0.01$: statistical significance as compared to controls (with or without diluent).

Ovine GH, LH and PRL radioimmunoassays

Ovine GH, LH and PRL were measured in duplicate by specific double antibody radioimmunoassays (RIA) [14, 15, 17]. Reagents were kindly provided by the National Hormone and Pituitary Program (NIDDK, Bethesda, Maryland) with the exception of the primary antibody for oPRL (#AR1.6) which was a gift from Dr H. Friesen (Winniepeg, Manitoba, Canada) [17]. The sensitivity of the assays was 0.4 ng(oGH-I-4)/ml, 0.2 ng(NIH-P-S12)/ml and 0.15 ng(NIH-LH-S24)/ml. Inter-assay variation was 5, 7 and 6%, respectively. Representative samples were prescreened at several dilutions, to test for parallelism to the standard curve, and then all samples from a specific experiment were run in the same assay at the appropriate dilution.

DNA and protein determinations

Four wells of cells were scraped and pooled for each determination of DNA and protein. The DNA was measured according to the method described by Erwin et al. [18] while the protein content was assayed using the method of Lowry et al. [19].

Expression of the results

GH, PRL and LH results are expressed as nanograms per ml of culture medium (Tables 1 and 2) or as a per cent of the appropriate control values (Figs 1–6); by normalising the data as a per cent of control, the results of several repeat experiments could be pooled. Because basal hormone secretion was not altered by the presence of diluent in the medium, all control (with or without diluent) data were pooled for each experiment prior to statistical analysis. DNA and protein results were expressed as µg/4 wells. GH, PRL and LH data were statistically analysed using analysis of variance (ANOVA) followed by Duncan's multiple range test after logarithmic transformation.

RESULTS

Acute effects of tamoxifen on GH

During acute (3 h) exposure of the pituitary somatotropes to increasing concentrations of tamoxifen (0.1–10 µmol/l), there was a dose-related inhibition of basal GH secretion (Fig. 1). The decrease was always significant ($P < 0.01$) for the highest dose tested (10 µmol/l); 1 µmol/l tamoxifen caused a significant inhibition ($P < 0.01$) during one of four experiments, while 0.1 µmol/l tamoxifen did not alter basal GH release.

10 µmol/l tamoxifen also significantly inhibited the GH response to 1 nmol/l GRF during an acute (3 h) incubation: there was a 69% decrease relative to the control-GRF group ($P < 0.01$) (Fig. 2; $n = 4$ experiments). The lower doses of

Table 2. Effect of chronic treatment with tamoxifen on 24 h secretion of (a) LH, (b) GH, and (c) PRL

	Time (h)		
	0	72	144
(a) LH			
Control (with or without diluent)	1.40 (0.11)*	0.60 (0.06)	0.49 (0.07)
Tamoxifen (1 μmol/l) 144 h	1.45 (0.19)	0.64 (0.08)	0.46 (0.04)
Tamoxifen (1 μmol/l) 72 h/Con 72 h	1.59 (0.19)	0.70 (0.05)	0.48 (0.01)
Tamoxifen (10 μmol/l) 144 h:		0.36 (0.02)†	0.32 (0.03)†
Tamoxifen (10 μmol/l) 72 h/Con 72 h	1.51 (0.12) 1.57 (0.18)	0.37 (0.02)†	0.45 (0.05)
(b) GH			
Control (with or without diluent)	236 (28)	127 (20)	49 (6)
Tamoxifen (1 μmol/l) 144 h	227 (21)	132 (17)	51 (4)
Tamoxifen (1 μmol/l) 72 h/Con 72 h	236 (12)	130 (2)	50 (8)
Tamoxifen (10 μmol/l) 144 h		31 (6)†	25 (2)†
Tamoxifen (10 μmol/l) 72 h/Con 72 h	250 (11) 254 (4)	31 (1)†	47 (4)
(c) PRL			
Control (with or without diluent)	217 (13)	192 (18)	111 (22)
Tamoxifen (1 μmol/l) 144 h	248 (24)	131 (6)†	53 (6)†
Tamoxifen (1 μmol/l) 72 h/Con 72 h	233 (21)	140 (8)†	96 (5)†
Tamoxifen (10 μmol/l) 144 h		< 14.5†	< 14.5†
Tamoxifen (10 μmol/l) 72 h/Con 72 h	212 (19) 216 (8)	< 14.5†	65 (7)†

* Data are expressed as ng/ml, mean (S.D.), $n = 4$ wells/experimental group.

† $P < 0.01$: statistical significance as compared to controls (with or without diluent).
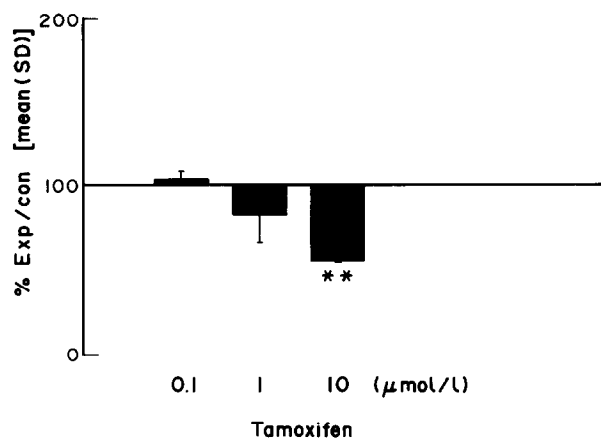

Fig. 1. Acute effects of tamoxifen on basal somatotrope activity. Results are expressed as a ratio of GH released during tamoxifen treatment (exp) over that released during control (with or without diluent) conditions (con). ** $P < 0.01$ as compared with the control (con/con) group of data.
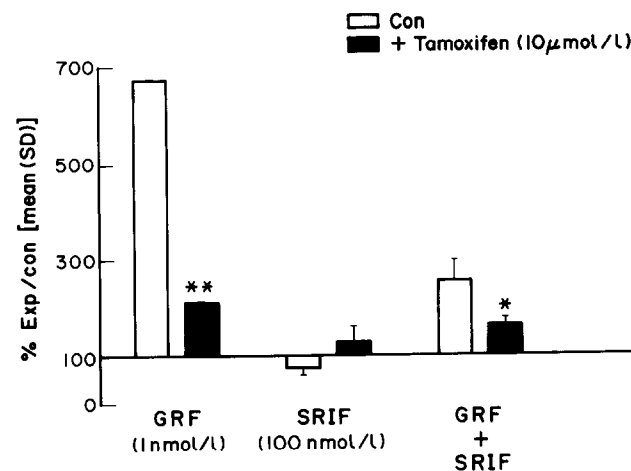

Fig. 2. Acute effects of tamoxifen during 3 h GRF-stimulated and/or somatostatin-inhibited GH release. Results are expressed as a per cent of their respective control (with or without 10 μmol/l tamoxifen). * $P < 0.05$, ** $P < 0.01$ as compared with the tamoxifen-untreated (open bar) group. SRIF = somatostatin.
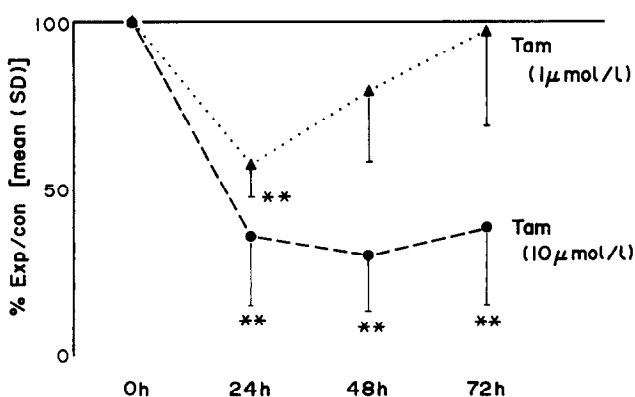

Fig. 3. GH secretion during 72 h exposure to tamoxifen. Results are expressed as a per cent of control GH secretion during the same 24 h period. ** $P < 0.01$ as compared with the tamoxifen-untreated group.

tamoxifen (0.1 or 1 μmol/l) had no consistent effect (data not shown).

100 nmol/l somatostatin caused a 30% decrease in 3 h GH secretion under control (with or without diluent) conditions; none of the tamoxifen doses significantly altered this inhibitory effect (Fig. 2; data not shown). While the data in Fig. 2 (as well as in later figures) suggest that 10 μmol/l tamoxifen had the tendency to block the actions of somatostatin, these results reflect the fact that the data are expressed as experimental values over their respective controls: this high dose of tamoxifen exerts, by itself, a maximal inhibitory effect on basal GH release (Fig. 1).

The 10 μmol/l dose of tamoxifen also appears to be maximally inhibitory for GRF-stimulated GH release; when 10 μmol/l tamoxifen was added in the presence of both GRF and somatostatin, the decrease in GH secretion was not significantly different from that observed with GRF alone (Fig. 2). As a result, the apparently significant ($P < 0.05$) difference between the two
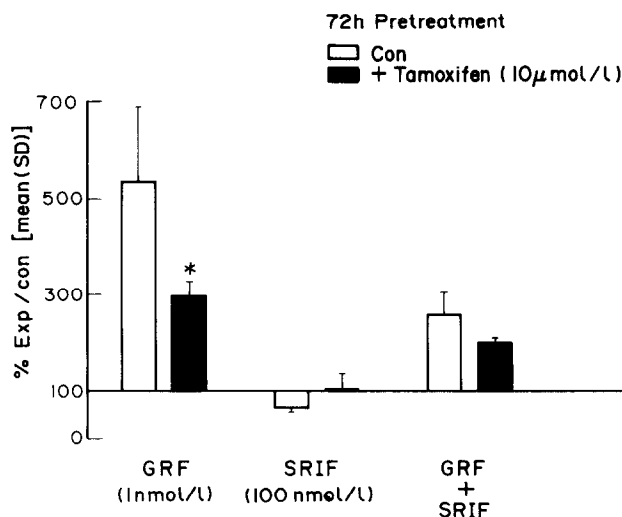
Fig. 4. Effect of 72 h of pretreatment with tamoxifen on subsequent 3 h GRF-stimulated and/or SRIF-inhibited GH release. Results are expressed as a per cent of their respective control (with or without pretreatment with tamoxifen). * $P < 0.05$ as compared with the tamoxifen-untreated (open bar) group.

Fig. 6. 3 h GRF-stimulated and/or somatostatin-inhibited GH release following: (1) 144 h pretreatment with tamoxifen (solid bars) or (2) 72 h pretreatment with tamoxifen followed by 72 h in control medium (hatched bars). Results are expressed as a per cent of their respective control (with or without pretreatment with tamoxifen). ** $P < 0.01$ as compared with the tamoxifen-untreated (open bar) group.

GRF plus somatostatin groups due to tamoxifen treatment is not of physiological importance (Fig. 2).

Chronic effects of tamoxifen on GH

Chronic exposure of the somatotropes to tamoxifen caused a dose-related decrease in daily GH secretion (Fig. 3; $n = 6$ experiments). The inhibition was significant for the 10 μmol/l dose over a 1–6 day period [35.8 (19.1)% mean (S.D.) of control; $P < 0.01$] (Figs 3 and 5; data not shown). 1 μmol/l tamoxifen consistently decreased GH release for the first 24 h [57.7 (10.0); $P < 0.01$]; however, by 48 h its action was less pronounced and by 72 h basal secretion had returned to control values (Fig. 3). 0.1 μmol/l tamoxifen had no significant effect at any of the time points examined.

Following 72 h incubation with 10 μmol/l tamoxifen, there was a significant decrease in the subsequent 3 h basal [67 (6)% of control; $P < 0.01$] as well as 1 nmol/l GRF-stimulated secretion of GH ($P < 0.05$) (Fig. 4; $n = 3$ experiments). As observed previously, during the acute treatment experiments,
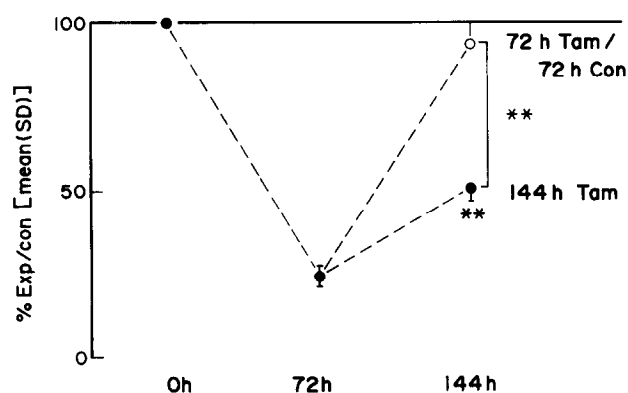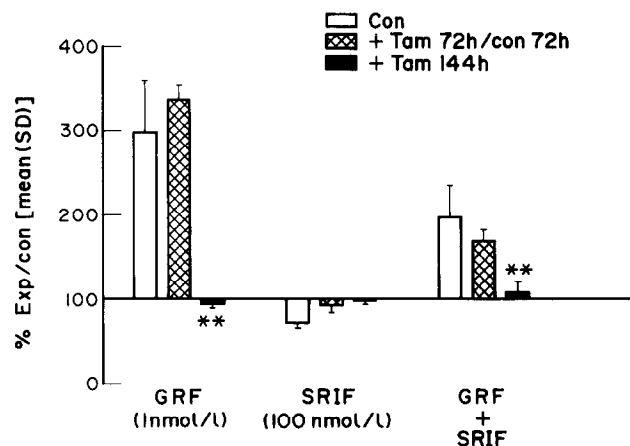

Fig. 5. GH secretion during either continuous exposure to tamoxifen over 144 h or 72 h exposure to tamoxifen followed by 72 h in control medium. Results are expressed as a per cent of control GH secretion during the same 24 h period. ** $P < 0.01$ as compared with the tamoxifen-untreated group. ⌐ ** $P < 0.01$, statistical difference between the two experimental groups.

there was no significant change in the somatotropes response to 100 nmol/l somatostatin. Also, pretreatment with the two lower doses of tamoxifen had no consistent effect on the subsequent 3 h basal, GRF-stimulated or somatostatin inhibited GH release (data not shown).

In one experiment, cultures were treated with 10 μmol/l tamoxifen for 72 h and then divided into two experimental groups: one set continued to be treated with 10 μmol/l tamoxifen for a further 72 h, while the second set was placed in control medium for 72 h, to determine whether the cells could recover from the drug treatment (Fig. 5; $n = 1$ experiment). These groups underwent a 3 h test with GRF with or without somatostatin following the first 72 h, and again following the second 72 h (Fig. 6).

In those cultures that were treated with 10 μmol/l tamoxifen for a total of 144 h, daily GH secretion continued to be markedly suppresed ($P < 0.01$) (Fig. 5). In addition, response to 1 nmol/l GRF was now completely blocked ($P < 0.01$) (Fig. 6, solid bars). The chronic tamoxifen treatment again had no significant effect on somatostatin (Fig. 6).

Those cultures exposed to 10 μmol/l tamoxifen for 72 h followed by 72 h in control medium showed a total recovery of daily basal GH secretion (Fig. 5). In addition, there was complete recovery of a subsequent 3 h response to 1 nmol/l GRF alone or the combination of GRF plus somatostatin (Fig. 6).

Acute and chronic effects of tamoxifen on ovine PRL

Tamoxifen had profound dose-related, inhibitory effects on lactotrope function in this test system. Both the 1 and 10 μmol/l doses significantly decreased PRL release under acute (Table 1; $n = 1$ experiment) as well as chronic (Table 2; $n = 1$ experiment) conditions; the 10 μmol/l dose suppressed PRL secretion to below RIA detectability after 3 and 6 days of exposure. When cultures were allowed to recover for 72 h following 72 h of tamoxifen treatment (Table 2), lactotrope activity returned to control values only in those cultures pretreated with the 1 μmol/l dose of tamoxifen; those cells pretreated with the 10 μmol/l dose continued to secrete PRL at a rate significantly lower than the controls ($P < 0.01$).

Acute and chronic effects of tamoxifen on ovine LH

The gonadotropes were relatively unaffected by exposure to tamoxifen. LH release remained unchanged during acute tests (Table 1), while only the highest dose of the anti-oestrogen significantly inhibited LH following chronic treatment ($P < 0.01$) (Table 2). Those cells allowed to recover from 72 h exposure to 10 μmol/l tamoxifen returned to control LH values (Table 2).

Chronic effects of tamoxifen on DNA and protein

None of the doses of tamoxifen tested had any significant effect on cell DNA or protein following chronic (3 or 6 day) treatment. Control cultures had 7.4 (1.9) μg DNA and 127 (18) μg protein/4 wells ($n = 3$ experiments); cultures treated with 0.1–10 μmol/l tamoxifen had 8.9 (3.2) μg DNA and 174 (38) μg protein/4 wells.

DISCUSSION

Tamoxifen is an effective hormonal agent in both adjuvant and palliative treatment of breast cancer [1, 20]. The classic mechanism of action of this drug is thought to involve a blockade of oestrogen receptors in tumour cells [8, 21]. However, our investigations suggest that tamoxifen may also be reducing tumour growth through suppression of the GH-IGF-I axis: (1) tamoxifen inhibits both basal and GRF-stimulated GH release from pituitary cells (present study); (2) serum levels of (GH-dependent) IGF-I are lower in breast cancer patients receiving tamoxifen than those receiving placebo [6]; and (3) inhibition of IGF activity at the level of the breast cancer cell is associated with decreased growth of the mammary tumour [3]. Thus, tamoxifen may decrease proliferation of breast cancer cells both directly, at the tumour level, and indirectly, by suppressing pituitary somatotrope function.

Our present experimental model is one that we have been using for several years to study mechanisms that regulate GH secretion. It is similar to the system established by Jordan and his colleagues to do comparative testing of anti-oestrogens in that it consists of primary monolayer cultures of immature pituitary cells; our source is the lamb while they use the rat [8, 14, 15]. One major difference between these two test models, besides the obvious one of species, is that we have made no attempt to remove oestrogens from the culture environment. Our goal in this initial series of experiments was to determine whether tamoxifen could modulate GH secretion under conditions that were as physiological as possible, not to determine the exact mechanism(s) by which tamoxifen might exert its effects.

In our test system, tamoxifen suppressed basal somatotrope activity following both acute and chronic exposure, indicating that this drug may interfere with mechanisms that regulate hormone synthesis as well as secretion. In addition, the anti-oestrogen may be down-regulating the GRF receptor or at least compromising its functional activity: the longer the pretreatment period, the greater the inhibitory effect of the 10 umol/l dose of tamoxifen on somatotrope responsivness to GRF.

In contrast, tamoxifen did not appear to enhance the inhibitory effects of somatostatin on basal or GRF-stimulated GH release. It is possible, however, that if we were to change the test conditions and use a lower than maximal dose of somatostatin as well as doses of tamoxifen between 1 μmol/l (that gave an inconsistent inhibition) and 10 μmol/l (that gave a maximal inhibition), we might observe an interaction between these two inhibitory factors. This possibility is of importance in light of a recent suggestion that the combination of tamoxifen and a long-acting somatostatin analogue be evaluated in clinical trials of breast cancer patients [6, 22]. Previous proposals to test the efficacy of somatostatin analogues have been based on the finding of somatostatin receptors in a population of breast cancers, as well as on studies showing that somatostatin has direct anti-proliferative effects on tumor cells [23, 24]. Since IGF-I is a potent mitogen for breast cancer, the reduction in IGF-I levels seen with somatostatin analogues [25] or anti-oestrogen treatment [6, 7] may be therapeutically relevant; and a combined anti-oestrogen and somatostatin treatment may reduce IGF-I levels further than is observed with either agent alone.

It is unlikely that the suppressive effects of tamoxifen on pituitary cell function were secondary to drug cytotoxicity. There was a significant inhibition of both GH and PRL secretion within as short a period of exposure as 3 h. Moreover, even after 6 days of treatment, those cells incubated with the highest dose (10 μmol/l) of tamoxifen had the same morphological appearance under phase microscopy as cells in control medium and there were no significant changes in culture DNA or protein levels. In addition, 72 h following 10 μmol/l tamoxifen withdrawal, secretion of GH and LH had returned to normal while PRL release showed almost 60% recovery. Also, tamoxifen seemed to have a differential rather than global effect on the three pituitary cell types, the lactotropes being more susceptible than somatotropes, and both were more sensitive than the gonadotropes.

The most effective concentration of tamoxifen in our system was 10 μmol/l: this dose consistently inhibited both basal and stimulated somatotrope activity. Evidence that such an effect has biological significance includes: (1) this is only three times the highest steady state serum level observed in patients on chronic tamoxifen therapy [16]; and (2) unlike under *in vivo* conditions, in our cultures there is probably no transformation of the tamoxifen to its 10-fold more potent metabolite, 4-hydroxy tamoxifen [8].

Whether tamoxifen is inhibiting somatotrope activity by binding to oestrogen receptors can not be concluded from our experimental protocols since we did not compare tamoxifen actions in the presence versus absence of oestrogens. Certainly, oestrogen receptors have been identified on somatotropes [13] and there are a large number of clinical as well as experimental data to support the concept that sex steroids, especially oestrogens, are an important physiological modulator of GH secretion. Both spontaneous as well as stimulated GH peak levels increase during puberty and decrease after the second decade, while postpubertal females have higher mean serum GH concentrations than age-matched males; these changes in somatotrope activity are strongly correlated with circulating levels of oestrogens but not testosterone, PRL or cortisol [9]. In addition, changes in the gonadal steroid environment, due to pharmacological manipulation, results in parallel changes in GH secretion [10, 11]. Similar data have been reported from animal studies, including both indirect *in vivo* and direct *in vitro* experiments [12, 13].

Despite the fact that GH has been implicated in breast tumour growth, its functional role is not clearly understood [1]. *In vivo* experiments have shown that high doses of GH can influence mammary growth and tissue IGF-I levels in oestrogen-treated animals, but a direct GH-induced mitogenic effect has been difficult to demonstrate under *in vitro* test conditions [1, 26]. GH receptors have been identified in several breast cancer cell lines and, more recently, mRNA for the GH receptor was

localised to the alveolar epithelial cells of the normal bovine mammary gland; however, binding studies using tissue membranes have revealed little to no specific GH binding [1, 27]. Thus, published data suggest that the major physiological role for GH is via regulation of hepatic IGF-I production and circulating IGF-I levels.

IGF-I is considered to be a potent mitogen for breast tumour cells [1-3, 5]. Recent data suggest that both serum and local tissue IGF-I may be involved in breast cancer growth; tumour tissues contain IGF-I mRNA and, using *in situ* hybridisation techniques, this mRNA has been localised to the mammary stromal (but not normal or malignant epithelial) cells [4]. What influences this local production of IGF-I is not yet known. However, it is clear, from *in vivo* as well as *in vitro* experiments, that inhibition of IGF-I biologic activity (by blocking the Type I IGF receptor) results in decreased mammary tumour growth [5]. Therefore, decreasing circulating as well as local tissue production of IGF-I may offer an important new therapeutic strategy for breast cancer.

In summary, our *in vitro* studies demonstrate that tamoxifen has direct effects on pituitary function, inhibiting GH, PRL and, to a lesser extent, LH secretion. These data, in combination with the results of our clinical investigations, suggest that tamoxifen may exert its anti-neoplastic effect by suppressing the pituitary-IGF-I axis as well as by blocking oestrogen receptors directly on the tumour cells. Further experiments are needed to determine whether: (1) tamoxifen decreases somatotrope activity through an oestrogen or a non-oestrogen receptor pathway, (2) tamoxifen alters GH synthesis as well as secretion, and (3) tamoxifen modulates somatotrope responsiveness to GRF at the GRF receptor or a secondary intracellular level.

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