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The biological activity of a single dose of tamoxifen in the adult ovariectomized rat

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1 The peripheral and central activities of tamoxifen were studied in the ovariectomized adult rat, up to 16 d after a single dose of 7.0 or 0.7 mg/kg.

2 Food consumption and body weight were decreased; only food consumption returned to control values after 16 d.

3 The weights of the uterus and of the uterine luminal fluid were increased for up to 8 d.

4 Serum follicle-stimulating hormone (FSH) concentrations decreased, but only significantly 1 and 8 d after 7.0 mg tamoxifen/kg. Serum luteinizing hormone (LH) and prolactin concentrations were elevated for up to 4 d.

5 Lordosis behaviour was absent throughout the period studied.

6 Within 3 d of administration, tamoxifen partially antagonized the oestrogen-induced changes in uterine luminal fluid, prolactin and LH secretion and lordosis behaviour.

7 Tamoxifen did not alter oestrogen-induced changes in uterine weight, food consumption and body weight.

8 The experiments demonstrate that tamoxifen is active for up to 16 d after a single intraperitoneal dose; oestrogen agonist, partial agonist and antagonist activities were demonstrated. The duration and type of activity depends upon the dose of tamoxifen and the target tissue response examined.

Introduction

Oestrogen antagonists have become important tools for the investigation of oestrogen action and have found a role clinically in the treatment of anovulation and breast cancer (Lunan & Klopper, 1975; Heel, Brogden, Speight & Avery, 1978; Furr, Patterson, Richardson, Slater & Wakeling, 1979). Consequently the drugs have been the focus of much research. Animal models used for analysis of the effects of the drugs usually involve changes produced upon the oestrogen-stimulated vaginal epithelium or uterus of the rat or mouse (see Dorfman, 1969). Endogenous oestrogen secretion must be kept low to minimize interference in the assay, and to avoid ovariectomy of the adult animal, the oestrogen-deficient prepubertal rat has become established in recent years (Dorfman, 1969; Jordan, 1979; Black & Goode, 1980). Compounds examined in this model have shown both oestrogen antagonist and oestrogen agonist activity (Dorfman, 1969; Katzenellenbogen, 1979; Wakeling & Slater, 1981) and such results are often used to describe the activity of a drug upon other oestrogen target systems and also as terms of reference in mature animals without further experimentation. However, recent results would suggest that there are differences in the response to oestrogens and oestrogen antagonists involving the age of the animal and the target system employed (Black & Goode, 1980; Bowman, Leake & Morris, 1982a). The pharmacology of oestrogen antagonists has also been complicated by suggestions that multiple doses of the compounds are necessary in order to detect biological activity (Clarke & Peck, 1979) and that the drugs may eventually be metabolized to other potent oestrogen antagonists (Hayes, Rorke, Robertson, Katzenellenbogen, & Katzenellenbogen, 1981; Wakeling & Slater, 1981).

The oestrogen antagonist, tamoxifen, will combine with oestrogen receptors prepared from a wide varie-

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ty of tissues and we have recently described the hypothalamic, pituitary gland and uterine receptor changes and serum concentrations of tamoxifen and metabolites after a single dose administered to the adult ovariectomized rat (Bowman, Leake & Morris, 1982b). The present study describes the biological activity of a single dose of tamoxifen in the adult ovariectomized rat upon central and peripheral oestrogen target tissues. Besides helping to establish a relationship between biological activity and the oestrogen receptor changes, the results provide information for the mature animal.

Methods

Adult female Sprague-Dawley rats (200–250 g) bred in the Medical School were bilaterally ovariectomized 21 d before the experiments. Tamoxifen (ICI Ltd., (Pharmaceuticals), Macclesfield, Cheshire), progesterone and oestradiol benzoate (Sigma [London] Chemical Co., Poole, Dorset), were injected, dissolved in arachis oil (1 ml/kg). Control rats received arachis oil alone (1 ml/kg). The temporal effects of tamoxifen (i.p.) were examined in rats maintained in normal lighting (light on 07 h 00 min–19 h 00 min) in groups of 8 with food and water *ad libitum*, except when body weight, food intake and sexual behaviour were measured. To determine these parameters, rats were housed in pairs in reversed lighting (lights on 20 h 30 min–10 h 30 min), and allowed access to 50 g food daily. Sexual behaviour was assessed in these rats before and 4–6 h after progesterone (4 mg/kg s.c.). Rats were killed by decapitation up to 16 d after treatment.

In experiments designed to investigate the anti-oestrogenic effects of tamoxifen, rats were housed individually in reversed lighting. On d − 2 they were allowed access to 50 g food daily, body weight and food consumption being measured daily. Where appropriate, the following treatments were tested: arachis oil or tamoxifen injected on d 0, followed on d 1 by subcutaneous injection of either arachis oil or oestradiol benzoate (100 μg/kg). On the morning of d 3 progesterone (4 mg/kg) was administered subcutaneously to all rats. Sexual behaviour was estimated 4–6 h after progesterone. The rats were killed by decapitation 0.5–1.0 h after the behavioural test.

In both experiments blood was collected, serum prepared and stored at −15°C until assayed. Before removal, the uterine horns were ligated *in situ* at ovarian and cervical ends, so that the wet weight and luminal fluid could be determined.

Behavioural tests were carried out in semi-circular cages (diameter 1 m) under red light. The number of lordoses displayed by the female in response to ten mounts achieved by two adult male rats housed in the test cage was determined. Testing of responsive females was complete within 5 min. Unresponsive females vigourously fought the sexually active male and the male was also less attracted to these females, so the behavioural test was often extended to between 20 and 30 min. The results are expressed as a percentage lordosis quotient, i.e. No. of lordoses ÷ No. of mounts × 100.

Gonadotrophin estimations were determined by radioimmunoassay, using reagents supplied by NIAMDD, NIH, Bethesda, Maryland, U.S.A. Details of the assays are given in Bowman, Leake, Miller & Morris, (1981), with the exception that the reagents used to assay serum prolactin in the serum up to 16 d after tamoxifen were changed to antisera S–7 and reference preparation RP-2. The within-assay coefficients of variation for luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin I (antisera PRL-13; reference preparation RP-I) and prolactin II (antisera S-7 and reference preparation RP-2) were 12, 10, 18 and 17% respectively. The sensitivities of the assays for LH, FSH, prolactin I and prolactin II were 10, 50, 0.5 and 0.1 ng/ml respectively.

The results are expressed as the mean ± s.e.mean. Parametric statistics were used to compare treatments, except for the hormone and behavioural data which are considered not to be normally distributed, therefore non parametric tests are employed as follows: Table 1, the temporal effects of tamoxifen were subjected to the following statistical analyses. With the exception of food and body weight data, 1 d and 16 d control values were compared, using Student's *t* test for the uterine weight and uterine luminal fluid results, and Mann-Whitney U test for the hormone data. There was no significant difference between 1 d and 16 d control rats for uterine and hormonal measurements, apart from an increase in serum LH ($P < 0.01$), therefore statistical comparisons have been made with the 1 day arachis oil control except for serum LH concentrations where comparisons have only been made between treatments at day 1 and day 16. Food intake and body weight changes were compared between treatments, and uterine weight and uterine luminal fluid within treatments by analysis of variance (One Way), followed by Dunnets' test for multiple comparisons with a control group. The temporal changes in FSH and prolactin concentrations and between treatments at 1 and 16 d for LH concentrations were compared by Kruskal-Wallis analysis of variance followed by Mann-Whitney U test. Table 2, the effects of tamoxifen upon arachis oil and oestradiol benzoate-treated rats were compared within treatments, and oestradiol benzoate compared with arachis oil-treated rats by the appropriate analysis of variance parametric or non parametric procedure as outlined for Table 1.

Table 1 Changes induced by tamoxifen (T 0.7 mg/kg and 7.0 mg/kg) in oestrogen target systems in the ovariectomized adult rat.

		Days after treatment 1		2		4		8		16	
Change in body weight (g)	C	1.8±1.0	(12)	3.6±0.8	(12)	2.1±1.3	(12)	5.9±1.8	(12)	13.0±1.4	(12)
	T0.7	−1.5±1.0*	(12)	−6.3±1.0**	(12)	−13.5±1.0**	(12)	−13.2±1.3**	(12)	−3.5±1.7**	(12)
	T7.0	0±1.1	(12)	−4.0±1.0**	(12)	−11.1±1.6**	(12)	−16.2±2.1**	(12)	−4.5±2.4**	(12)
Total food intake (g)	C	19.5±1.2	(6)	17.6±1.0	(6)	14.6±1.0	(6)	17.3±0.3	(6)	19.1±0.9	(6)
	T0.7	10.7±0.8**	(6)	9.0±1.1**	(6)	8.7±0.4**	(6)	11.6±0.3**	(6)	17.3±1.3	(6)
	T7.0	14.0±0.8*	(6)	13.5±0.8**	(6)	12.1±0.9*	(6)	12.4±0.7**	(6)	16.6±1.2	(6)
Uterine weight (mg)	C	89±4	(18)	—		—		—		91±4	(12)
	T0.7	130±5††	(16)	152±5††	(20)	134±4††	(20)	116±4††	(18)	98±5	(18)
	T7.0	149±6††	(8)	162±7††	(8)	127±10††	(8)	142±5††	(8)	97±6	(8)
Uterine luminal fluid (mg)	C	10±1	(18)	—		—		—		9±1	(12)
	T0.7	15±1††	(16)	19±1††	(20)	15±2†	(20)	11±1	(18)	8±1	(18)
	T7.0	18±1††	(8)	23±1††	(8)	21±1†	(8)	17±1†	(8)	10±1	(8)
Serum FSH (ng/ml)	C	1980±87	(23)	—		—		—		2076±109	(20)
	T0.7	1744±115	(15)	1830±163	(13)	1929±118	(15)	1816±122	(12)	1812±134	(15)
	T7.0	1672±165†	(15)	2025±216	(16)	1856±92	(15)	1614±67††	(17)	1745±219	(12)
Serum LH (ng/ml)	C	114±10	(30)	—		—		—		185±12	(20)
	T0.7	113±15	(12)	112±12	(13)	89±13	(10)	121±15	(8)	186±17	(9)
	T7.0	200±19**	(15)	177±13	(15)	174±8	(16)	137±9	(18)	130±10*	(16)
Serum prolactin (ng/ml)	C	2.26±0.23	(30)	—		—		—		3.42±0.85	(18)
	T0.7	5.2±1.2†	(8)	3.8±1.2	(9)	3.1±0.7	(8)	3.3±0.6	(6)	2.9±0.4	(7)
	T7.0	8.06±1.94††	(13)	5.59±1.11†	(14)	6.34±1.58††	(15)	3.21±0.56	(17)	4.92±1.12	(12)

Tamoxifen was administered as a single i.p. dose and the rats examined up to 16 d later. Control (C) rats received arachis oil (1 ml/kg).
Values are mean ± s.e.mean. The number of observations is given in parentheses.
$P<0.05$*, <0.01** v control.
$P<0.05$†, <0.01†† v 1 day arachis oil control.
For details of the statistical analyses see Methods.

Results

Temporal changes

The results obtained up to 16 d after a single dose of tamoxifen 0.7 or 7.0 mg/kg, are given in Table 1. Both doses of tamoxifen caused a fall in body weight which was maximal between d 4 and 8; thereafter the rats gained weight but control values were not achieved by 16 d. Food intake was also suppressed, returning to control values by 16 d. It should be noted that the food intake in the control rats was variable, so that it is not possible to make accurate statements about the length of time food intake was changed.

Uterine weight increased to a maximum at 2 d and remained elevated up to 4 and 8 d after tamoxifen 0.7 and 7.0 mg/kg respectively; similar increases were seen in the accumulation of luminal fluid.

Serum LH concentrations were difficult to evaluate, as the 16 d control value was significantly greater than the 0 d control. Only the higher dose of tamoxifen significantly increased this gonadotrophin concentration after 1 d and possibly up to 4 d after administration. In contrast, after 16 d its concentration in the tamoxifen-treated rats was lower than controls. Prolactin concentrations in the serum were increased in an apparently dose-related manner. The decrease in serum FSH concentrations seen after tamoxifen is small and only achieved statistical significance 1 and 8 d after 7.0 mg tamoxifen/kg. However, no rat showed the lordosis response throughout the experimental period, even after prior progesterone treatment.

Oestrogen antagonist activity

The effects of tamoxifen, oestradiol benzoate and a combination of the two drugs are presented in Table 2. Oestradiol benzoate was administered 24 h after tamoxifen at which time the receptor changes induced by the latter were near to maximum (Bowman *et al.*, 1982b) and the animals killed 48 h later. The changes produced by tamoxifen itself are similar to those described in the previous section and are not discussed further. Oestradiol benzoate induced

Table 2 Oestrogen antagonism by a single dose of tamoxifen in the adult ovariectomized rat

Oestrogen treated	*Oestradiol benzoate plus tamoxifen (mg/kg)*		
	0	0.7	7
Change in body weight (g)	−5.9 ± 1.9*	−14.8 ± 2.0	−9.4 ± 2.1
Total food intake (g)	45.9 ± 2.7*	26.4 ± 1.9	34.6 ± 2.1†
Uterine wt. (mg)	202 ± 9.5**	214.8 ± 15.5	194.1 ± 27.0
Luminal fluid (mg)	308 ± 33 **	195.4 ± 40.6	75.5 ± 64.4††
Lordosis quotient %	99*	90	58††
Serum FSH (ng/ml)	705 ± 37	758 ± 58	651 ± 25
Serum LH (ng/ml)	2663 ± 369**	1904 ± 666	565 ± 128††
Serum prolactin (ng/ml)	201 ± 13 **	216 ± 15	118 ± 28†
Number of determinations	8	8	5
Control		*Tamoxifen (mg/kg)*	
	0	0.7	7
Change in in body weight	2.6 ± 2.6	−8.8 ± 3.1*	−10.3 ± 1.6*
Total food intake (g)	52.8 ± 3.1	44 ± 2.7	40.2 ± 2.9*
Uterine wt. (mg)	92.7 ± 7.3	118 ± 14.8	152.2 ± 8.7**
Luminal fluid (mg)	10.2 ± 0.7	9.9 ± 0.9	12.0 ± 0.8
Lordosis quotient %	0	0	5
Serum FSH (ng/ml)	733 ± 27	695 ± 53	626 ± 47
Serum LH (ng/ml)	645 ± 119	681 ± 100	777 ± 148
Serum prolactin (ng/ml)	26.5 ± 2.4	37.2 ± 9.2	42.0 ± 11.8
Number of determinations	5	6	6

Values are mean ± s.e.mean.
$P < 0.05$*, < 0.01** v control–Tamoxifen 0 mg/kg
$P < 0.05$†, < 0.01†† v oestradiol benzoate–Tamoxifen 0 mg/kg
For details of the statistical analyses see Methods

changes in body weight and food intake similar to those produced by tamoxifen. However, uterine luminal fluid, uterine weight and sexual behaviour were all greatly increased by oestradiol benzoate over both control and tamoxifen groups. Oestradiol benzoate raised serum LH and prolactin concentrations but FSH concentrations were unchanged.

Tamoxifen did not antagonize the response to oestrogen in terms of body weight, food intake, serum FSH concentration and, surprisingly, uterine weight. However, the accumulation of uterine luminal fluid and the raised serum LH concentration produced by oestradiol benzoate were inhibited in a dose-related manner. Partial antagonism of the effects of oestradiol benzoate upon serum prolactin and sexual behaviour was produced by the high dose of tamoxifen 7.0 mg/kg.

Discussion

Tamoxifen acted as an oestrogen agonist in terms of the regulation of body weight and food intake and this was manifest after both tamoxifen alone or in combination with oestrogen; in this respect, the action is similar to the oestrogen antagonists, clomiphene and nafoxidine (Wade & Blaustein, 1978; Bowman *et al.*, 1981). Bowman *et al.* (1981) showed that this activity of clomiphene was clearly dose-related, but in the present study with tamoxifen, it was not. The mechanism by which oestrogen and the oestrogen antagonists inhibit food intake and growth is not understood, one suggestion being mediation via the oestrogen receptor system of the hypothalamus. Wade & Blaustein (1978) found a correlation between hypothalamic oestrogen binding and suppression of food intake. High affinity oestrogen receptor concentrations after tamoxifen have recently been described and the receptor concentrations return to control values before control values of food intake and body weight are re-established (Bowman *et al.*, 1982b). The lowest dose of tamoxifen used in this study does not affect the hypothalamic receptors (Bowman *et al.*, 1982b), yet changes in food intake and body weight are comparable at both doses, suggesting that these activities are unrelated to the high affinity oestrogen receptors, a view we have reached using the oestrogen antagonist clomiphene (Bowman *et al.*, 1981).

The partial oestrogen agonist and antagonist activity of multiple doses of tamoxifen upon the immature rat uterus is well documented (Furr *et al.*, 1979;

Jordan, 1979; Black & Goode, 1980). The present study in the adult rat also demonstrates that tamoxifen will promote the growth of the uterus and the accumulation of luminal fluid, although not so efficiently as oestrogen. The maximal effect of tamoxifen was observed after 2 d and at this time the oestrogen receptor concentrations of the tissue nuclei are at their highest (Bowman *et al.*, 1982b). Uterine growth is not sustained and after this time, both uterine weight and nuclear oestrogen receptor concentrations decline, approaching control values between 8 and 16 d. Koseki, Zava, Chamness & McGuire (1977) have also demonstrated prolonged uterotrophism associated with raised nuclear oestrogen receptors after tamoxifen.

The neuroendocrine changes after tamoxifen are complex. Ovariectomized rats were used so that the endogenous oestrogen receptor mechanisms would be free to react with tamoxifen. Up to 16 d after tamoxifen, FSH secretion was only marginally changed. Serum prolactin concentrations were increased in an apparently dose-related manner, being sustained for up to 4 d after tamoxifen 7.0 mg/kg. These effects upon pituitary hormone secretion are similar to those found for clomiphene (Bowman *et al.*, 1981). LH secretion was initially increased by tamoxifen, but 16 d after administration was unexpectedly lower than in control animals. FSH and LH secretion is controlled by the hypothalamus and anterior pituitary gland whilst previous receptor data (Bowman *et al.*, 1982b) indicate that tamoxifen will act upon both these tissues. Therefore the present results in the ovariectomized adult rat indicate that tamoxifen does not directly affect FSH secretion. LH secretion was increased after the high dose of tamoxifen when oestrogen receptors of both hypothalamus and pituitary gland were occupied by the ligand. It is tempting to suggest that LH secretion is stimulated by tamoxifen acting upon the hypothalamus to promote LHRH secretion, but this would not account for the observation that LH secretion is significantly inhibited 16 d after tamoxifen administration.

In the ovariectomized rat lordosis is absent and not restored by treatment with a variety of oestrogen antagonists (Etgen, 1979). However, after clomiphene, if the period of testing is extended lordosis behaviour develops 7–9 d later (Bowman *et al.*, 1981). Unlike clomiphene, tamoxifen failed to restore any lordosis behaviour in tests carried out up to 16 d.

In our examination of the oestrogen antagonist activity of tamoxifen, oestrogen was given at the time tamoxifen induced its maximum receptor occupancy. In spite of large changes in uterine receptor concentrations, tamoxifen was unable to antagonize the uterotrophic response to oestrogen, although partial antagonism of the luminal fluid response was seen. This result is similar to that reported for clomiphene in the adult rat and contrasts with the oestrogen antagonism reported (after multiple doses) in the immature rat which can be demonstrated before there are marked changes in receptor populations (Jordan, Dix, Rowsby & Prestwich, 1977). Antagonism of oestrogen by cyclofenil, tamoxifen and trioxifene is more clearly demonstrable in the immature rodent (Black & Goode, 1980; Bowman *et al.*, 1982a). Thus the uterine tissues of the adult and immature animal appear not to be comparable and results ought not to be extrapolated between animals of these different age groups.

Oestradiol benzoate treatment increased serum LH concentration, which is attributable to the initiation of a positive feedback mechanism (Brown-Grant, 1976). Tamoxifen (7.0 mg/kg) antagonized this action, in agreement with the demonstration of a decreased ovulation rate after tamoxifen (Labhsetwar, 1970) which is an index of LH secretion. The lower peripherally active dose of tamoxifen did not inhibit oestrogen-induced LH secretion, so that the origin of the positive feed back appears to be within the central nervous system.

Tamoxifen is used for the treatment of breast cancer (Heel *et al.*, 1978; Furr *et al.*, 1979). A substantial proportion of the tumours are hormonally dependent, oestrogen and perhaps prolactin being considered to play a promotional role in their growth (Nagasawa, 1979; Nisker & Siiteri, 1981), therefore it is desirable to maintain oestrogen and prolactin serum concentrations as low as possible. In the present experiments, tamoxifen increased prolactin secretion, but to a lesser extent than oestradiol benzoate. Tamoxifen only partially antagonized the oestrogen-stimulated prolactin secretion. The reason for this is unclear since even multiple doses have also been reported to be inefficient in this respect (Jordan & Koerner, 1976). It cannot be attributed to the use of the ovariectomized rat since similar findings apply using intact normal and tumour-bearing rats (Nicholson & Golder, 1975; Jordan & Koerner, 1976; Nagy, Valdenegro & MacLeod, 1980). The significance of this result, in experimental animals and when oestrogen antagonists are used for the treatment of breast cancer in humans remains to be evaluated. In contrast, clomiphene completely reverses oestrogen-induced prolactin secretion (Bowman *et al.*, 1981).

In conclusion, the present experiments have shown that agonist activity of tamoxifen upon oestrogen target tissues can be detected up to 16 d after a single injection. The oestrogen antagonism of a single dose of tamoxifen in the adult rat, examined at a time of maximal receptor occupancy was also demonstrated. However, the magnitude of these changes is varied and their relationship to the oestrogen receptor is complex and must be considered for each target

system. The prolonged and multiple changes induced by tamoxifen in the adult female rat imply the need for caution when extrapolating results, from the immature rat uterus using multiple doses, to other oestrogen target organs, experimental models or clinical situations.

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