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Pituitary-related weight changes affecting the liver, uterus and adrenal glands of rats treated with hexoestrol and clomiphene in high doses

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Summary

The synthetic oestrogen hexoestrol, administered at 60 mg/kg/day for 4 days to female rats in 3 studies, caused the folowing mean changes in the relative weights of some of the principal organs: liver (+37%), spleen (−11%), adrenals (+43%), kidneys (+3%), pituitary (+23%), uterus (+49%), and ovaries (+13%). The heart weights showed no consistent changes. The mean relative organ weights of hypophysectomized, hexoestrol-treated rats did not differ significantly from those of untreated hypophysectomized controls. The latter animals had lower organ weights than sham-operated controls. Pretreatment with clomiphene citrate at dose levels of 20—60 mg/kg/day prevented in a dose-dependent manner most of the organ weight changes induced by hexoestrol. The exception was the adrenal weight, which was increased. Compared with controls, the mean relative organ weights of rats receiving clomiphene alone at 60 mg/kg/day differed as follows: liver (−18%), spleen (−17%), heart (−16%), adrenals (+7%), kidneys (−11%), pituitary (−13%), uterus (−25%), and ovaries (−4%). The changes affecting the liver, spleen, heart, kidneys and uterus were significant. Rats immunised with a monkey antiserum to rat growth hormone and treated with hexoestrol had significantly lower relative liver weights than did animals treated with hexoestrol alone. No other significant differences were observed. It is tentatively concluded that there is a mediating or co-operative pituitary influence involved in the significant hepatic, uterine and adrenal weight gains caused by hexoestrol. Clomiphene may act centrally to affect these organ weight changes and, indeed, may act at this level to (mainly) anti-organotrophic effect even in the absence of exogenous oestrogen. In the case of the hexoestrol-induced liver enlargement, the role of pituitary growth hormone is worth further investigation.

Key words: Oestrogen; Anti-oestrogen; Organ weights; Hypothalamic-pituitary axis; Uterus; Liver

Introduction

Female rats given the non-steroidal oestrogens hexoestrol and diethylstilboestrol at 60 and 6 mg/kg/day, respectively, in the course of a 42-day

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toxicity study, displayed increased pituitary, adrenal, uterine, ovarian and hepatic weights [5]. Such organotrophic effects are commonly observed in the rat after high-dose administration of oestrogens, whether synthetic or natural, steroidal or non-steroidal [7,23]. Clomiphene citrate at a pretreatment dose level of 2 mg/kg/day acts anti-oestrogenically to reduce the uterine and pituitary weight gains caused by diethylstilboestrol at 6 mg/kg/day, while exaggerating the adrenal enlargement [5]. The questions arise: How do oestrogens, administered in 'toxicological' doses, exert their organotrophic effects? And how does clomiphene act to influence these effects?

A pituitary contribution to oestrogen-induced organ weight changes has been reported for the rat liver [2], adrenals [20] and uterus [10]. Accordingly, the present studies focused on the role of the pituitary, and in particular on that of a hypophysial hormone of known anabolic properties, growth hormone.

This side-investigation of a toxicity study comprised three experiments in which were used the endocrine techniques of (putative) anti-hormone intervention, surgical ablation and immunisation with antiserum. The results support the tentative identification of a pituitary involvement in hexoestrol-induced hepatic, uterine and adrenal weight gains in the rat.

Materials and methods

Animals

In the studies reported here there were 5 adult female rats in each experimental group. They were Wistar albinos (University of Surrey stock, unless otherwise specified). The animals in each group were housed in polycarbonate cages and maintained at a temperature of 22 ± 2°C and a relative humidity of 50 ± 5%. They were fed on Spratt's Laboratory Diet No. 1, Expanded (Spratt's Patent Ltd., Barking, Essex, U.K.), and were permitted unrestricted access to this and water.

Chemicals

Hexoestrol (4,4'-dihydroxy-α,β-diethyldiphenylethane) was purchaesd from Sigma Chemical Co. Ltd. (Poole, Dorset, U.K.), and clomiphene citrate (2-[*p*-(2-chloro-1,2-diphenyl-vinyl)phenoxy]triethylamine dihydrogen citrate) (mixed isomers) was a generous donation by Dr J.D.F. Lockhart of Merrell Division, Richardson-Merrell Ltd. (Slough, Berkshire, U.K.). The monkey antiserum to rat growth hormone was generously supplied free under the National Institute of Arthritis, Metabolism and Digestive Diseases (NIAMDD) Rat Pituitary Hormone Distribution Program, U.S.A., courtesy of Dr A.F. Parlow of Harbor General Hospital (Torrance, CA, U.S.A.).

Protocol

Hexoestrol was administered at 60 mg/kg/day and clomiphene citrate at 20—60 mg/kg/day (see Fig. 1 and Table I). Both compounds were dissolved in isopropyl myristate (myristic acid isopropyl ester; Sigma) and administered orally by gastric intubation needle. The pretreatment compound or drug vehicle, in the

case of controls, were given 1 h before the treatment compound or a second dose of drug vehicle. The dosing period in each of the experiments was 4 days. The animals were weighed at the start and end of this period at the same time of day. At the termination of the studies the rats were anaesthetized with ether and exsanguinated from the inferior vena cava. The following organs were removed in a standard order, trimmed free of connective tissue and fat, and weighed: liver, spleen, heart, adrenals, kidneys, pituitary, uterus and ovaries. Before being weighed, the uteri were drained of intraluminal fluid by slitting the uterine horns, followed by gentle blotting. The relative organ weights were expressed as a percentage of body weight.

Statistical analysis

The usual formula for standard deviation was used in the statistical analysis, together with Student's *t*-test.

Experiment 1

Clomiphene was used in this experiment in conformity with a previous protocol [5]. This compound is known to compete with oestrogen for the high-affinity intracellular receptors through which the hormone is believed to act, in the uterus and elsewhere [22]. Thirty adult female rats in the weight range 190—245 g were assigned to six groups as shown in Fig. 1.

Experiment 2

Hypophysectomized and sham-operated (i.e. intact) female rats were used to assess the role of the pituitary in hexoestrol-induced organ weight changes. They were Wistar albinos (Crl:(WI)BR) supplied by Charles River U.K. Ltd. (Margate, Kent, UK). The operative procedures were carried out by the supplier and this experiment was performed within 3 weeks of surgery. Hypophysectomy caused a virtual cessation of growth. This and the 3 week post-operative period account for the differences in the initial body weights of the hypophysectomized and sham-operated rats, animals of the same age. There were 4 experimental groups as shown in Table I.

Experiment 3

This experiment was directed towards defining the involvement, if any, of pituitary growth hormone in hexoestrol-induced organ weight changes. To this end a monkey antiserum to rat growth hormone was used to neutralise the biological activity of the endogenous hormone. The use of an antiserum for this purpose is well established [6]. Oestrogens are known to increase plasma levels of growth hormone in the female rat while decreasing the storage of this hormone in the pituitary [1], and growth hormone has been shown to cause visceral enlargement (including liver growth) when administered to rodents [12]. Enlargement of the soft tissues is also observed in man in such disorders as gigantism and acromegaly, and this is usually attributed to an excess of growth hormone.

Twenty adult female rats in the weight range 190—220 g were assigned to 4 groups as shown in Table I. The monkey antiserum to rat growth hormone was

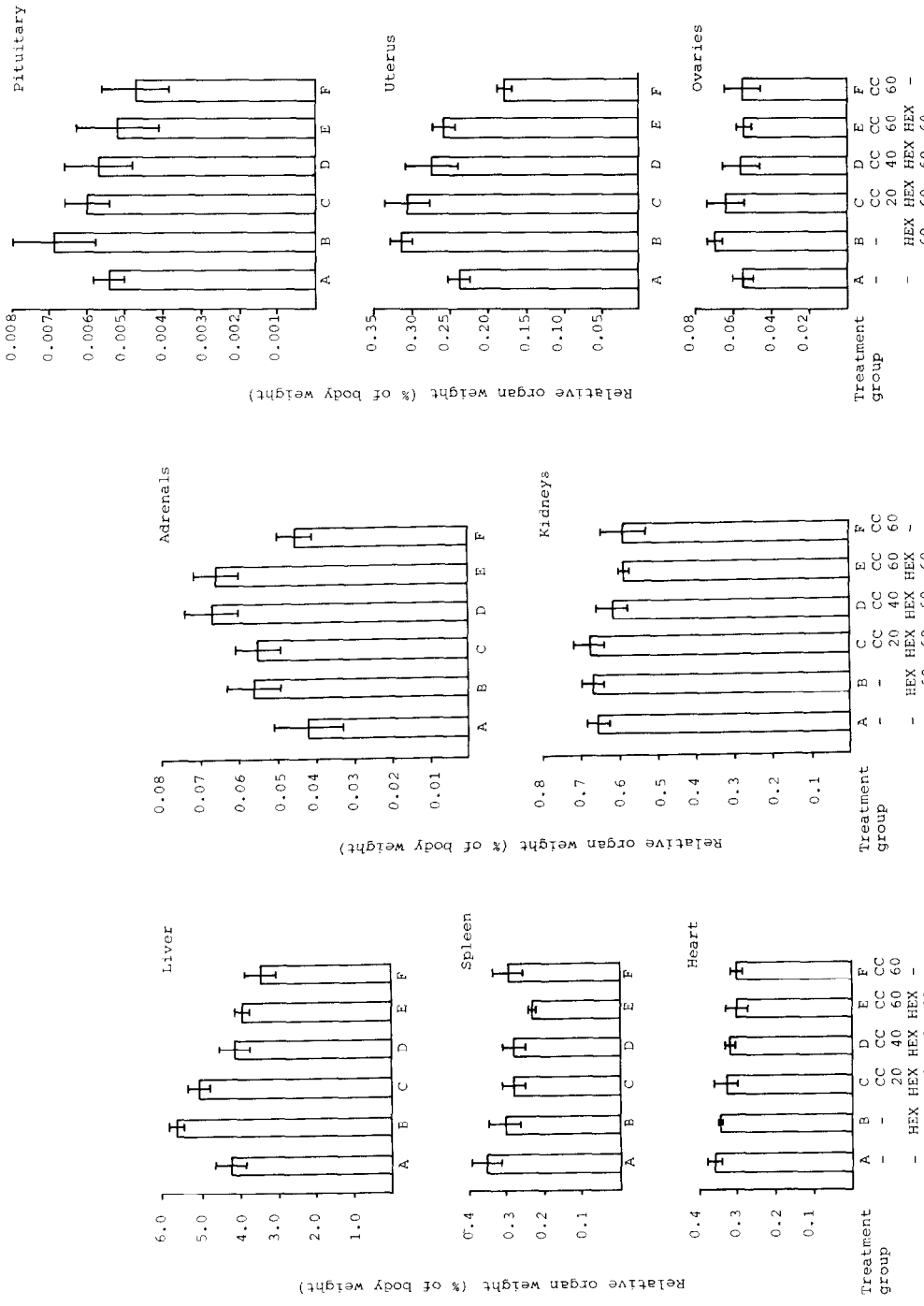

Fig. 1. (a—c) Relative organ weights of female rats treated with hexoestrol and clomiphene citrate, and of controls: 6 treatment groups, A—F; HEX = hexoestrol; CC = clomiphene citrate; figures give dose rate, mg/kg/day. Each column represents mean ± S.D. of data from 5 animals. Study period: 4 days.

TABLE I

BODY WEIGHTS (g) AND RELATIVE ORGAN WEIGHTS (%)

Treatment group	Body weight		Liver	Spleen	Heart	Kidneys	Adrenals	Ovaries	Uterus	Pituitary
	Initial	Final								
Experiment 2. Hypophysectomy										
A	224	225	3.60	0.33	0.34	0.64	0.037	0.061	0.196	0.0038
Intact	± 9	± 7	± 0.11	± 0.03	± 0.03	± 0.04	± 0.003	± 0.010	± 0.043	± 0.0002
B	223	216	5.13	0.28	0.34	0.66	0.055	0.066	0.317	0.0046
Intact	± 8	± 9	± 0.30	± 0.01	± 0.03	± 0.07	± 0.009	± 0.006	± 0.033	± 0.0005
HEX										
C	189	181	3.18	0.21	0.28	0.46	0.015	0.034	0.139	NA
Hypox	± 6	± 5	± 0.27	± 0.02	± 0.02	± 0.04	± 0.001	± 0.003	± 0.030	
HEX										
D	183	178	2.85	0.21	0.28	0.48	0.014	0.034	0.136	NA
Hypox	± 2	± 1	± 0.20	± 0.03	± 0.01	± 0.02	± 0.002	± 0.003	± 0.024	
Experiment 3. Monkey antiserum to rat growth hormone										
A	202	203	3.79	0.27	0.34	0.58	0.036	0.058	0.190	0.0054
Controls	± 5	± 4	± 0.22	± 0.02	± 0.05	± 0.01	± 0.002	± 0.009	± 0.034	± 0.0002
B	203	205	5.20	0.26	0.35	0.61	0.054	0.062	0.291	0.0065
HEX	± 7	± 7	± 0.29	± 0.03	± 0.06	± 0.05	± 0.007	± 0.012	± 0.041	± 0.0009
C	202	205	4.69	0.26	0.34	0.55	0.049	0.062	0.324	0.0074
Anti-GH	± 6	± 9	± 0.25	± 0.05	± 0.05	± 0.03	± 0.008	± 0.008	± 0.026	± 0.0009
HEX										
D	202	210	3.84	0.28	0.35	0.58	0.030	0.050	0.203	0.0053
Anti-GH	± 10	± 8	± 0.27	± 0.02	± 0.01	± 0.04	± 0.003	± 0.002	± 0.044	± 0.0003

HEX = hexoestrol (60 mg/kg/day); Hypox = hypophysectomized; Anti-GH = monkey antiserum to rat growth hormone; NA = not applicable. Figures represent mean ± S.D. of data from 5 animals. Study period: 4 days.

diluted (1 in 60) with physiological saline and administered by intravenous injection into the tail vein at 1 ml/kg/day. The antiserum or, in the case of controls, saline, was administered immediately before the oestrogen or dosing vehicle.

Results

Hexoestrol and clomiphene

In Experiment 1, body weight changes, where these occurred, were insufficient to complicate the organ weight analysis. By group, the initial and final body weights (g) ± S.D., were: A, 209 ± 7, 213 ± 12; B, 201 ± 8, 208 ± 13; C, 235 ± 8, 238 ± 14; D, 223 ± 9, 219 ± 9; E, 235 ± 8, 215 ± 6; F, 238 ± 5, 238 ± 4.

In the rats treated with hexoestrol alone (Group B), compared with controls, there were significant increases in the relative weights of the liver ($P < 0.01$), pituitary ($P < 0.05$), uterus ($P < 0.01$) and ovaries ($P < 0.02$) (Fig. 1).

Clomiphene administered alone (Group F) decreased most of the relative organ weights compared with controls. The direction, extent and significance of these changes were as follows: liver (−18%, $P < 0.05$), spleen (−17%, $P < 0.05$), heart (−16%, $P < 0.01$), kidneys (−11%, $P < 0.02$), and uterus (−25%, $P < 0.01$). The pituitary, ovarian and adrenal weights differed non-significantly from controls by −13%, −4%, and +7%, respectively.

The relative organ weights of the animals treated with both clomiphene and hexoestrol (Groups C, D and E) were generally lower than those of animals receiving hexoestrol alone (Group B), and in some cases were comparable to those of rats given clomiphene alone (Group F). Clomiphene antagonized in a dose-dependent manner the hexoestrol-induced increases in the liver, pituitary, uterus and ovaries while reducing, in a broadly dose-dependent manner, the spleen, heart and kidney weights. The mean relative adrenal weights of the Groups D and E rats were exceptional in being higher than those of the hexoestrol-only rats of Group B.

Pituitary

The intact controls (Group A) maintained their body weights over the 4-day study period in Experiment 2 (Table I). The rats of Groups B, C and D showed moderate reductions in weight. All the relative organ weights were lower in the hypophysectomized controls (Group D) than in their intact counterparts (Group A) (Table I). Hypophysectomy in the adult rat, besides leading to the arrest of body growth also gives rise to splanchnomicria [8].

In the intact rats of Group B, hexoestrol provoked significant increases in the relative weights of the liver ($P < 0.001$), adrenals ($P < 0.02$), pituitary ($P < 0.05$), and uterus ($P < 0.01$), compared with the intact controls of Group A, and a significant decrease in the spleen weights ($P < 0.05$). In contrast to this, hexoestrol caused no significant changes in the relative organ weights of hypophysectomized rats compared with those of untreated hypophysectomized controls.

Growth hormone

Most of the animals in Experiment 3 either maintained their body weights or

showed modest gains over the study period (Table I). Hexoestrol caused a significant increase in the relative weights of the liver ($P < 0.01$), adrenals ($P < 0.01$), pituitary ($P < 0.05$), and uterus ($P < 0.02$) (Table I). The organ weight changes seen in the hexosterol-treated rats (Group B) also occurred in those which received the growth hormone antiserum as well (Group C), the only significant difference being a lower liver weight gain ($P < 0.02$). There were no significant differences in organ weights between the rats which received the growth hormone antiserum alone (Group D) and controls (Group A).

Aggregated data

The mean differences in relative organ weights between hexoestrol-treated and control animals in the 3 studies taken together were as follows: liver (+37%), spleen (−11%), adrenals (+43%), kidneys (+3%), pituitary (+23%), uterus (+49%), and ovaries (+13%). The heart weights showed no consistent changes.

Discussion

Hexoestrol when administered in very high doses to the female rat caused reproducible alterations in the weights of several of the principal organs. Significant pituitary, uterine and hepatic weight gains were noted in all 3 experiments, while significant adrenal weight gains were noted in 2. A significant ovarian weight gain was noted in one experiment, and a significant splenic weight reduction in another. An essentially similar pattern of organ weight changes occurred in the 42-day toxicity study described above, in animals moreover which, unlike those in the present studies, showed significant and progressive reductions in body weight [4]. The main alterations in organ weights caused by hexoestrol are therefore consistent, sustained, and not artefacts of body weight changes.

Liver, uterine, pituitary, and adrenal weight gains are commonly observed in female rats given oestrogens in large doses [7,23]. The histological character of the liver weight gain has been determined in a study directly comparable to the present ones [4], and consists of cellular hypertrophy, particularly around the centrilobular veins, and hyperplasia, as evidenced by abundant mitotic figures. The uterine changes will probably have comprised hypertrophy and hyperplasia of the luminal epithelium, stromal hypertrophy and oedema, and myometrial hypertrophy [11]. An enlargement in pituitary size after high-dose oestrogen treatment usually aries from a proliferation of the chromophobe cells with a consequent decrease in the relative proportions of acidophils and basophils [5,14]. Cortical hypertrophy and hyperplasia probably accounted for the observed adrenal weight gains [5,7], but the histological background to the (generally non-significant) splenic and ovarian weight changes is unclear.

Pituitary involvement

Experiment 2 indicated that hypophysectomy reduces or eliminates the significant organ weight changes induced by hexoestrol affecting the uterus, liver and adrenals. These findings confirm that in the rat an intact pituitary is necessary for full oestrogen-dependent growth of the uterus [10], adrenals [20] and

liver [2], but indicate that a mediating or co-operative pituitary influence operates even at the highest dose levels of oestrogen.

The uterine weight data obtained in Experiment 2 were somewhat unexpected: the uterine weights of hypophysectomized rats treated with hexoestrol were only 2% above control values compared with 62% in intact animals. Comparable investigations to the present one involving hypophysectomized but otherwise intact animals would appear to be lacking. Work on ovariectomized rats suggests that the pituitary contribution in question may be indirect, via TSH; the late phase of estrogen action seems to depend on the presence of thyroid hormones [3]. The uterotrophic response is not significantly augmented in hypophysectomized rats by separate injections of ovine prolactin, follicle stimulating hormone, luteinizing hormone, or growth hormone [21]. The non-involvement of this last-mentioned hormone is supported by Experiment 3 which involved an antiserum to growth hormone. This study, though, provided preliminary evidence that the hexoestrol-induced liver weight gain in the rat may be due in some degree to an indirect increase in the secretion of pituitary growth hormone.

Hypophysectomy abolished the customary adrenal weight gain seen in intact hexoestrol-treated animals (Experiment 2). The pituitary influence indicated by this result may be a feedback-induced increase in ACTH secretion caused by a fall in free corticosteroid secondary to an oestrogen-elicited rise in corticosteroid-binding globulin [18].

Clomiphene

The increases in the organ weights observed when hexoestrol was administered alone were largely prevented in a dose-dependent manner by pretreatment with clomiphene. The exception was the adrenal weight (see below). A similar, though less marked, effect has been observed in rats treated with both diethylstilboestrol and clomiphene at dose levels of 6 mg/kg/day and 2 mg/kg/day, respectively [5]. Clomiphene has been reported previously to inhibit oestrogen-induced growth of the rat pituitary [17] and rat uterus [15]. In the mouse it has been shown to inhibit completely the hepatotrophic effects of a single 2 mg/kg dose of oestradiol undecylate; a significant increase in liver weight and cell-count, and in hepatic content of protein, DNA and RNA, can be prevented by clomiphene citrate administered for 7 days at 0.5 mg/kg/day [19].

Granted a mediating or co-operative pituitary role in hexoestrol-induced uterine, hepatic and adrenal weight gains, it can be speculated that the observed effects of clomiphene are due to competitive antagonism at some point along the hypothalamic-pituitary axis. This would imply that clomiphene acts centrally to prevent, for example, the possible increase in growth hormone secretion implicated in the hexoestrol-induced liver enlargement. A single site of action would account for the rather similar dose-dependent pattern of stepwise decrements in organ weights seen in Experiment 1 (Fig. 1). Clomiphene depresses the uptake of oestradiol by the anterior pituitary and anterior hypothalamus [9].

It was a somewhat unexpected finding that clomiphene adminstered alone decreased most of the organ weights. The depressant effect of high doses of

clomiphene has been reported for the rat pituitary, uterus, and ovaries [13], and for the mouse liver [19], but significant changes in the rat liver, spleen, kidneys and heart have not been described. In reducing the size of the viscera, clomiphene treatment resembled in character, though not in degree, the effects of hypophysectomy. This might be regarded as circumstantial evidence that clomiphene is acting centrally. Antagonism of endogenous oestrogen may be involved here, as clomiphene is ineffective in reducing the liver weight in ovariectomized mice [19].

In clomiphene-treated animals, the mean weight of the adrenals was the only one above control values. Furthermore, clomiphene exaggerated the enlargement provoked by hexoestrol in the adrenal weights, which was in contrast to its effects on the other hexoestrol-induced weight changes. On the basis of histological evidence, it has been deduced that ACTH secretion is unaffected by clomiphene [25]. The present findings call this supposition into question, while also indicating differences between the oestrogen-related influences on adrenal size and those on the sizes of the other organs.

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